Memoirs of the Torrey Botanical Club

Volume 19 Number 1

Editor
M. A. Chrysler

The cytology of the Chytridiales with special reference to Cladochytrium replicatum

By

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Published for the Club by
GEORGE BANTA PUBLISHING COMPANY
MENASHA, WISCONSIN
1937

Botany
Direct
7-13-37

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JOHN S. KARLING

(WITH SIX PLATES AND TWO TEXT-FIGURES)

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I. CLADOCHYTRIUM REPLICATUM KARLING

INTRODUCTION

The species, genera, and families of fungi which are collectively grouped under the order Chytridiales are unusually diverse in structure, life cycles, habitat and method of development; and for a group of organisms which show so many widely different indications as to origin, descent, and phylogenetic relationships they have received comparatively little attention from cytologists. The majority of students of these fungi have for the most part been concerned with recording the occurrence of old and new species throughout the world and describing their developmental stages. Furthermore, the larger number of such descriptions have been only fragmentary and based on isolated stages of development, and have thus left wide gaps in our knowledge of the life cycles of the chytrids. This scarcity of cytological data is primarily due without doubt to the fact that the majority of the species are markedly evanescent and difficult to procure in sufficient quantities for intensive investigation, as well as unusually minute in size. In addition, very few of them are economically important as parasites, and have thus attracted but little attention from pathologists.

A general survey of the literature of the Chytridiales shows that scarcely more than six genera and less than two dozen species have so far been studied cytologically with any degree of intensity. As a consequence, very little is known about the details of karyokinesis, cytokinesis, asexual and sexual reproduction, pathogenicity, host and parasite relationship, etc., as a whole in the widely divergent families of the group. Meiosis or reduction division, for example, has been described in only one species, while chromosome counts have been made in only six. Furthermore, the accounts of these various cytological processes which have been given in the literature are directly conflicting in many instances, while others are scarcely more than a revival in modified form of the old theories of Schleiden and Schwann as to the origin of the nuclei and cell division.

The first part of this contribution will deal with the processes of nuclear and cell division, zoosporogenesis, resting spore development, and structure and organization of the thallus of *Cladochytrium replicatum* Karling, while the second will review the accounts of these processes as they are given in the literature of the Chytridiales as a whole. In the latter, the literature is quite extensive, and for the sake of convenience it may perhaps be best summarized and analyzed chronologically by separate families. The Plasmodiophoraceae are excluded from this discussion on the

ground that there is little or no agreement among students of the so-called Archimycetes as to their exact relation to the chytrids. The order in which the families are presented is not to be interpreted as indicating the author's conception of their phylogeny and relationship. This analysis is not primarily concerned with these problems, and for this reason the order of presentation is immaterial. Furthermore, a number of genera included here are very doubtful as far as their validity and relation to the chytrids are concerned, but in view of the fact that our knowledge of this group of fungi is at present so imperfect and fragmentary it is impossible to establish well-defined limits. While the majority of species and genera may possibly be grouped into vague and poorly delimited families, the order as a whole is scarcely more than a dumping ground for relatively simple fungi. At least it cannot be regarded as a phylogenetic group in our present state of knowledge.

MATERIALS AND METHODS

Among the various genera and species investigated by the author during the past ten years, none lends itself more favorably to cytological study than *Cladochytrium replicatum* Karling. Not only is it widely distributed in nature and thus readily available, but it can be easily grown in abundance as a saprophyte in sterilized plant tissues, thus eliminating most of the difficulties usually encountered in fixing, embedding, and staining these minute organisms. Furthermore, its nuclei and other cell organs are fairly large, clear and conspicuous in fixed and stained preparations. In this study *C. replicatum* was isolated from dead leaves of *Eriocaulon septangulare* and cultured in sterilized roots of *Panicum variegatum*, succulent internodes of *Tradescantia*, and cells of *Nitella flexilis*, as has been described in previous publications (1931b, 1935) by the author. These tissues were first boiled in water until fairly soft and then placed in small battery jars containing infected leaves of the pipewort. Within a few days they usually became heavily infected with the saprophyte, and by fixing such soft and fleshy tissues an abundance of thalli in all stages of development were secured. It is thus obvious that by this method *C. replicatum* can be manipulated as easily as root tips, anthers, etc., throughout the steps of killing, washing, dehydration, and imbedding.

A wide variety of fixing agents were employed, including Flemming's medium and weak solutions at full strength as well as in dilutions of $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, and $\frac{1}{10}$ with distilled water, chrom-acetic, Merkle's, osmic acid vapor, absolute alcohol, Bouin's, and Allen's and Wilson's modifications of Bouin's solution in various dilutions. Regaud's, Němec's, and Benda's fixatives were used for the study of chondriosomes and checked in each

case against intra-vitam staining with Janus green. Material killed in these latter solutions was stained in Heidenhain's iron haematoxylin, Benda's sulfalizarine crystal violet, and acid fuchsin-toluidin blue and aurantia, according to Champy-Kull's method. For the more general cytological structures Flemming's $\frac{1}{2}$ weak and $\frac{1}{4}$ medium solutions with a fixation period of 48 hours have given the best and most consistent results, particularly when followed by the triple stain. Likewise Allen's and Wilson's modifications of Bouin's solution when used in combination with Heidenhain's iron alum haematoxylin and counter-stained with dilute Orange G gave excellent preparations. Feulgen's nuclear and Newton's gentian violet stains were also employed to good effect, especially in relation to the origin and structure of the chromosomes. In all cases the appearance of the rhizomycelium, spindle organs, zoosporangia and resting spores in the fixed and stained preparations were continually compared with living and intra-vitam stained material, so as to check as far as possible the degree of alteration and artifact incurred.

METHOD OF INFECTION, DEVELOPMENT, STRUCTURE AND ORGANIZATION OF THE THALLUS

As has been described and figured in my previous papers on *C. replicatum*, the zoospores in the free swimming stage are uniciliate, hyaline, and spherical with a conspicuous, highly refractive, golden red globule lying in or near the center. This body frequently appears to lie in a vacuole, and is usually the only structure that can be seen with certainty in living material. In fixed and stained preparations, on the other hand, the appearance and structure are quite different. The refractive globule is no longer visible as such, and in the center of the zoospore lies a comparatively large nucleus with a small nucleole and a large densely stained external nuclear cap. The nucleus is generally connected with the point of attachment of the cilium by delicate and faint cytoplasmic strands.

Following the active swimming period the zoospore becomes sluggish and frequently amoeboid, loses its cilium and comes to rest on the host tissue, as is shown in figure 1. Very shortly afterwards, unless degeneration occurs, a very fine germ tube is produced which penetrates the wall of the host cell. The diameter and size of this tube depend to some degree on the character of the wall. If the host material has been cooked for a long time so that the walls are quite soft, the germ tube is relatively large, but when the wall is comparatively hard and compact the tube has an appearance like that shown in figure 2. Oftentimes under such circumstances the wall may appear slightly thicker in the region of penetration as if it has undergone local gelatinization. As soon as the tube has passed through the wall

it increases considerably in diameter, as is illustrated in figures 2 and 3. At this stage the nucleus and nuclear cap still lie in the spore body on the outside, and the cytoplasm usually appears more vacuolated. Shortly afterwards the nucleus migrates through the wall into the enlarged tube in the host cell. As is illustrated in figure 3, it appears extremely dense and heavily stained during this passage, and it is usually almost impossible to differentiate between the nucleole and nuclear cap. As to the history of the latter during this initial nuclear migration and the early developmental stages of the rhizomycelium, our knowledge at present is very fragmentary. In the majority of germinating zoospores so far observed very little remained behind in the external spore cases, and the suggestion becomes obvious that the nuclear cap passes with the nucleus into the germ tube. However, the fact that it is not usually evident and sharply defined in association with the nucleus in the incipient primary spindle organ, as is shown in figure 4, militates against this general assumption. It is not altogether improbable that it may be reserve food which is gradually used up in germination, although I have no concrete data in support of this hypothesis. If, as Debaisieux (1920) and Hatch (1935) claim, in *Coelomycidium Simulii* and *Allomyces arbuscula* it is formed by condensation and fusion of chondriosomes during the maturation and cleavage of the zoosporangium, it may break up into fragments and become dispersed in the elongating germ tube and young rhizomycelium as chondriosome *anlagen*. This again is purely hypothetical and I have so far failed to find any conclusive evidence to substantiate this supposition.

As the germ tube elongates it usually increases considerably in diameter near the tip, and a comparatively short spindle-shaped region is thereby formed. This is the rudiment of the initial spindle organ. The nucleus migrates into this region (fig. 4) and the first vegetative center of thallus replication is thus established. In a number of cases, however, no previous enlargement behind the tip of the germ tube has been found prior to the arrival of the nucleus. In such instances the tube merely enlarges in the region where the nucleus lies to form the spindle organ. As this goes on the elongated densely stained nucleus begins to shorten, vesiculate and round up, loses its intense chromaticity and assumes its characteristic appearance and structure. In this transformation, however, some changes apparently take place, because the nucleole emerges much larger in size than it was in the zoospore. The nucleus shown in figure 4 is considerably elongated and somewhat pear-shaped with the pointed end toward the old zoospore case, giving an appearance which suggests that it has but recently arrived in the incipient spindle organ. The nucleole is quite conspicuous and shaped like a broad flat ring lying around the inner periphery of the

pointed end. The chromatin is in the form of a faint reticulum and is not particularly clear and abundant. Such pear-shaped nuclei have often been encountered in young spindle organs, and from such preparations I have come to associate this peculiar shape with recent nuclear migration. The evidence for this, however, is more indirect and circumstantial than directly conclusive, as will appear shortly.

It is to be noted from the above description that in germination the center of gravity, so to speak, of growth, development, and organization is not retained in the spore, as in the families Synchytriaceae, Olpidiaceae, Woroninaceae, and some members of the Rhizidiaceae, but carried away to an outgrowth of it, the spindle organ. Up to this stage the thallus is monocentric in organization. After the first center has been established the thallus begins to extend rather rapidly. Rhizoids and branches begin to develop (fig. 4) and the spindle organ begins to increase in diameter. The nucleus keeps pace with its growth to a certain degree and then usually undergoes division as is shown in figure 5. This is followed very shortly by cell division, and the spindle organ becomes two-celled (fig. 6). One of the nuclei generally remains behind, while the other migrates out into the tenuous portion of the rhizomycelium and establishes a second center for growth and organization. In a few instances, however, both nuclei have been observed to travel on before cell division occurred, leaving the primary spindle organ empty.

Successive stages in the migration of the second nucleus from the initial spindle organ into the filamentous part of the thallus are shown in figures 6 to 11. The organ illustrated in figure 6 has undergone cytokinesis and is quite large. The lower nucleus has begun to enter the filament and is drawn to a sharp point at the apex. This portion is as densely stained as the nucleole, which can be clearly distinguished at one side of the nucleus as a somewhat hemispherical body. The cytoplasm toward the median wall has become more vacuolated. A slightly later stage perhaps is shown in figure 7. The apex of the nucleus lies in the filament and is drawn out into a dense pointed rod, while the nucleole and the remainder of the nucleus still lie in the spindle organ. The greater portion of the cytoplasm has also migrated with the nucleus and appears quite dense around it. Usually at this stage, the cytoplasm in the immediate vicinity of the nucleus is filled with round, irregular and angular, deeply stained bodies, which frequently tend to obscure the nuclear membrane. Figure 8 shows a nucleus in transit. It appears like a densely stained rod, slightly pointed at the apex and almost completely filling the filament. Quite often a break appears in it, as is shown so well in figure 8, which I have interpreted as a line of separation between the nucleole and the remainder of the nucleus. The nuclei shown

in figures 8 and 9 are rather short and thick, but it is not uncommon to find them more filamentous and drawn out. Figure 9 shows a nucleus about to enter the incipient secondary spindle organ, while in figure 10 is shown a full-grown organ of the same type in which the nucleus has become vesicular again and attained mature proportions. The nucleus in this latter figure is, none the less, still quite pointed at one end and markedly pear-shaped. From its pointed end a granular cytoplasmic strand runs through the organ into the filament. Such strands have frequently been observed, but their significance and functions are still obscure. They may possibly represent a line of flow or path along which the nucleus traveled, as Gilbert (1935) has suggested and illustrated in *Ceratiomyxa*. However, in a few spindle organs I have observed pear-shaped nuclei lying at right angles rather than parallel to the long axis, as in figure 10. The nucleole in this figure is again in the shape of a broad flat ring near the pointed end, while the chromatin reticulum is quite conspicuous and dense.

In figure 12 is shown a two-celled primary and an incipient unicellular secondary spindle organ together with some of the more filamentous portion of the rhizomycelium. One of the nuclei has migrated from the primary to the secondary spindle organ, while the other has remained behind and been blocked off by a cell wall. It is to be noted in this figure that the thallus has branched repeatedly and formed a number of rhizoids. The latter filaments are generally developed in abundance and are doubtless the chief organs of absorption. Structurally, they may often be of the same diameter and appearance as the main branch or axis, but very shortly run out to a fine filament and eventually end. Figure 11 also brings out sharply the wide variation in diameter and contour of the main axis and its branches. When first described by the author in 1931 from cells of *Eriocaulon septangulare* the filamentous portion of the rhizomycelium was reported to be very finely drawn out, and in the same year Sparrow described it (*C. Nowakowskii*) in several algae as "exceedingly tenuous, never attaining a diameter of more than 1.5 μ." However, when grown under the conditions used in this study the tenuous filaments may vary from 1.5 to 6 μ and sometimes become almost vesicular in certain regions, as is well illustrated in figure 12. Furthermore, the thallus spreads and proliferates so profusely that the host tissue may become filled with a meshwork of branches, rhizoids, spindle organs, zoosporangia, and resting spores. This is especially true in boiled internodes of *Nitella flexilis*, where the number of globular zoosporangia alone may run into thousands within a single cell.

The cytoplasm in the main axis, its branches and the rhizoids is usually quite vacuolated and contains a few scattered, densely stained fragments,

bodies, or granules of varying sizes and shapes. These stain rather intensely in all of the basic stains, and so far have shown no specific affinity for chondriosomal dyes. In the young spindle organs the cytoplasm is generally more dense, and the number of granules much greater. The latter are usually aggregated around the nucleus, suggesting perhaps a nuclear origin. Their appearance, size, and distribution will be discussed more in detail under the consideration of nuclear division. As the spindle organ matures and one or both nuclei migrate out, as has been noted above, the cytoplasm becomes more and more vacuolated and sparse and may often be reduced to a thin primordial utricle surrounding a central vacuole.

Following the stage illustrated in figure 12 the nucleus in the secondary spindle organ divides and one, and occasionally both, daughter nuclei migrate out into tertiary and quaternary spindle organs, and successively new centers of organization, growth, and development are thus established. In this fashion the thallus is continually reduplicated as it spreads in the host tissue. With the view of presenting more graphically the organization, type of development, and profuseness of the rhizomycelium of *C. replicatum*, I have drawn in plate 6 a portion of a thallus as it occurred in an internode of *Nitella flexilis*. This drawing is accurate as far as the general appearance, distribution, structure, and maturity of the thallus are concerned, but reconstructed with respect to the cytological details of nuclear migration, division, cleavage in the zoosporangia, etc. As will become apparent later, these details were added and the whole interior reconstructed from stages observed in other fixed and stained preparations in order to summarize more objectively in diagrammatic form the processes of nuclear and cell division and their relation to growth and development. The profuseness and variability in diameter of the rhizomycelium with its zoosporangia, spindle organs and resting spores are in no sense exaggerated in this figure. In fact many thalli have been found in internodes of *N. flexilis* which have been even more profuse and extended.

A careful analysis of this figure will emphasize the structural characteristics and organization of the thallus of *C. replicatum*. In the first place it is polycentric and continually replicated, and in this respect it is like the mycelium or thallus of the higher fungi. Spindle organs may follow spindle organs or alternate with tenuous filaments, zoosporangia, and, at maturity, with resting spores; and except for the rhizoids, the type of development is distinctly homeotic. In the early stages of growth the thallus is essentially trophocentric with the spindle organs as the vegetative centers of replication, but with the development of zoosporangia and resting spores at maturity it becomes more and more reproducto- or genocentric, as has been described in a previous paper (1932).

The second outstanding characteristic, and perhaps the most significant in comparison with the mycelium of the higher fungi, shown in plate 6 is that the nuclei are not regularly distributed along the length of the thallus, but are localized and confined to the spindle organs, zoosporangia, and resting spores. Nuclei may occasionally be seen in the filamentous portion of the rhizomycelium, but only in transit. As has been described above, they are migrating to new centers. This characteristic is of fundamental significance, it seems to me, from the standpoint of development and organization. In the thallus of the higher fungi the nuclei are distributed more or less regularly along the length of the mycelium, according to the figures in the literature, while in *C. replicatum* they are localized; and it is doubtless by virtue of this localization that the spindle organs become the vegetative centers of replication.

The rhizomycelium of this chytrid is, on the other hand, strikingly different from the thallus in the Synchytriaceae, Olpidiaceae, Woroninaceae, and Rhizidiaceae also. As has been previously described, the thalli of these four families are monocentric in contrast to the polycentricity of the Cladochytriaceae. This difference in structure and organization seems at present to be fundamentally and directly correlated with the extent of distribution of the nuclei in the thallus. In the first three families the entire thallus at maturity is holocarpic or transformed into zoospores, gametes or resting sporangia, while in the Rhizidiaceae there is considerable differentiation into vegetative and fertile portions. The vegetative parts may be limited to a simple unbranched absorbing organ as in species of *Phlyctidium* or greatly extended into a rhizoidal system as in *Diplophlyctis;* nevertheless, only a single zoosporangium, resting spore or center of organization is developed. This may be due to the fact that the nuclei remain in this center and never migrate out into the rhizoids, according to the figures in the literature. While the number of illustrations bearing directly on this point are limited (Lowenthal, 1905; Serbinow, 1907; Němec, 1912; Wager, 1913; Couch, 1935; Hovasse, 1936), they nevertheless show the nuclei as being confined to the reproductive portion of the thallus and never migrating into vegetative parts. In species of *Entophlyctis* and *Diplophlyctis* (Karling, 1930, 1931a) where the rhizoids may be as much as 6μ in diameter at their point of origin and extend over a radius of 700μ the author has never found nuclei except in the sporangia or resting spores. Monocentricity in organization appears to be thus directly correlated with localization in nuclear distribution. This is naturally to be expected since the nucleus is without doubt the dominating sphere of influence in metabolism, reproduction and organization.

In the family Cladochytriaceae on the other hand, as has been shown

by Tisdale, Jones and Dreschler, Bartlett and myself, the nuclei migrate out into the tenuous filaments, and where they come to rest new and successive centers of organization are formed. The thallus is thus continually replicated and becomes polycentric. The nuclei are nevertheless localized and confined to the turbinate and spindle organs during the strictly vegetative stage, and by virtue of this fact such structures become the centers of duplication. In *Physoderma Zeae-maydis*, *Ph. Menyanthis* (Sparrow, 1934), *Ph. maculare* and *Ph. pulposa*, however, evanescent monocentric rhizidiaceous zoosporangia are occasionally formed as in the family Rhizidiaceae, but unfortunately no cytological work has been done on such structures to show the distribution of the nuclei. In *Catenaria* sp., which the author is now studying cytologically, such monocentric thalli are frequently formed, and in all cases so far observed the nuclei are confined to the zoosporangia alone. In other cases where the successively divided nuclei migrate out into the filaments additional sporangia develop, and very shortly the thallus becomes polycentric. In this form it is thus possible to note the transition from monocentric to polycentric thalli and its relation to nuclear distribution.

Thus as far as our knowledge of the chytrids extends at the present time, the rhizomycelium of *C. replicatum* stands midway in organization between the mycelium of the higher fungi and the thallus of the Rhizidiaceae. On one hand it possesses rhizoids like species of the latter family, and on the other it is polycentric and continually replicated like a true mycelium. These similarities together with the fundamental differences noted above are justifications, in my opinion, for designating the thallus of this chytrid and most known members of the family Cladochytriaceae as a rhizomycelium. Quite recently, however, Bessey (1935, pp. 45-46) has given the impression that the term is to be applied only to the enucleate filamentous portion, but such a limitation is incorrect and does not conform to the author's original and present intention. The term applies to the thallus as a whole and includes the spindle organs, zoosporangia, resting spores, and rhizoids as well as the more tenuous part.

While the rhizomycelium appears at the present time to occupy a position more or less midway between the rhizoidal system and a true mycelium as far as structure, method of development and organization are concerned, there is no good evidence to indicate that it represents an evolutionary transitional type of thallus. It is to be particularly noted in this connection that *Physoderma* and *Catenaria* sometimes form evanescent monocentric thalli with rhizoids as in the family Rhizidiaceae, and the suggestion immediately arises that this is the more primitive type of development which is occasionally recapitulated in the life cycles of typically

rhizomycelioid species; and, furthermore, that the Cladochytriaceae have probably evolved from this family through the migration of the nuclei into the filamentous rhizoids and the acquisition of a polycentric mode of development. This conception, however, is purely hypothetical, and there are no concrete data to support it. As used in this and my previous papers the term rhizomycelium carries no phylogenetic connotation, and is primarily employed to emphasize some of the fundamental differences in structure and organization that exist among the various chytrids. Many mycologists make no distinction between the round protoplasts or plasmodium-like thalli of the Olpidiaceae, Woroninaceae and Synchytriaceae, the rhizoids of the Rhizidiaceae and the mycelium of the higher fungi, and designate them all by the latter term. Schwartz and Cook (1929), for example, speak of the oval thallus of *Olpidium radicale* as "a definite mycelium . . . . from which sex organs are formed . . . . ," while Fitzpatrick (1930) describes the rhizoids of the Rhizidiaceae as a mycelium regardless of whether they are minute, single, absorbing, or anchoring pegs as in *Phlyctidium brevipes* or greatly branched systems as in *Diplophlyctis*. Such broad and general use of terms, it seems to me, robs them of their significance. In the Chytridiales striking differences in structure, development, and organization do exist, and wherever possible they should be clearly recognized and properly designated.

Cladochytrium replicatum is primarily a saprophyte and does not develop in normal, healthy growing cells as far as my observations go. Consequently it is impossible to determine its effect on the nucleus and cytoplasm of the host cells. It is obvious from the methods employed during this study in preparing the host tissues for inoculation and infection that the cellular content is dead, badly distorted and frequently quite macerated in appearance. Thus the most conspicuous changes which appear in infected cells are not due to the presence of the fungus. Sparrow (1931) reports that *C. replicatum* (*C. Nowakowskii*) is a virulent parasite of *Spirogyra*, but I have so far failed to find it as such. In fact, as has been shown in a previous paper by the author (1935), it is very widely distributed and ubiquitous in nature and may infect the dead fleshy tissues of almost any plant.

NUCLEAR AND CELL DIVISION IN THE SPINDLE ORGANS

The nucleus of *C. replicatum* is quite large, well defined and shows definite structural characteristics as is shown in figure 13. Except when in transit it is usually spherical, oval, or somewhat citriform. Its most conspicuous element is a large nucleole which generally lies at one side near the nuclear membrane and stains intensely dark in the ordinary basic dyes.

The nucleole varies considerably in size and shape from a broad round band around the inner periphery of the nucleus (figs. 12, 15 16) to a more or less flattened spherical disk (figs. 17, 18, 22, 27, 32) or an oval body as in figures 13, 21, and 24. In the majority of nuclei studied it appears homogeneous throughout and even in contour, but occasionally it may be irregular, variable in density, and seemingly composed of a number of granules, as is suggested in figure 20. Whether or not in such exceptional cases this appearance is correlated with prophase nuclear changes has not been determined, but it seems unlikely. In preparations which have been poorly fixed and stained, the nucleole appears to lie in a conspicuous central vacuole, and is often the only visible structure in the nucleus, but such appearances are largely due to artifact.

In addition to the nucleole a definite chromatin reticulum or network is usually clearly visible in the resting nucleus, as is well illustrated in figures 13 to 16. In median sections of the nucleus, the reticulum appears denser and more abundant at the nuclear membrane, and in exceptional cases may leave the center almost devoid of chromatin (fig. 13). It is usually quite irregular in appearance, and seems to be made up of chromatin granules, net knots, and irregular bodies distributed on a linin framework. In good triple-stained preparations it stains deeply blue or violet in contrast to the ruby red nucleole, which thus suggests a fundamental difference in composition from the latter. The presence of this well defined chromatin reticulum in the resting nucleus militates against the general assumption and contention that the nucleole in the Chytridiales is the storehouse of chromatin and ultimate source of the chromosomes.

Quite often in the spindle organs and particularly in the young zoosporangia and resting spores the cytoplasm surrounding the nuclear membrane is filled with densely stained globules and granules of various sizes and shapes as is shown in figures 14 to 20; and it is not uncommon to find them in such abundance that the membrane is almost completely obscured and indistinguishable. In such cases the nucleus usually stands out in sharp contrast as a comparatively clear oval or circular region with the deeply stained nucleole at one side (fig. 33). These granules may be round, angular, or elongated and extremely variable in size. Figures 17, 18, and 26 show them as quite large, while in figure 24 they are of more or less uniform size and evenly distributed. In some spindle organs they may be lacking entirely, as is shown in figures 29, 32, 34, 35, and 36. It is primarily because of these granules that it is so difficult to determine with certainty the presence and structure of centrosomes and astral rays. Such granules are very common in the cells of the chytrids, and to date have been described in *Synchytrium* (Stevens and Stevens, 1903; Kusano, 1907; Perci-

val, 1910; Curtis, 1921; Kohler, 1923; Quintanilha, 1926), *Olpidium* (Kusano, 1912; Schwartz and Cook, 1928), *Polyphagus* (Wager, 1913; Dangeard, 1900), *Olpidiopsis* (Barrett, 1912), and *Urophlyctis* (Fron and Lasnier, 1920; Jones and Dreschler, 1920; Bartlett, 1926). Their source and manner of origin are not clearly known, but the majority of students of *Synchytrium*, *Olpidium*, and *Polyphagus* describe them as originating from the nucleole and being extruded from the nucleus preparatory to and during division. In the resting spores of *Polyphagus Euglenae* Wager (1913) regards them as chromidia or tropho-chromatin which represent the vegetative element in the nucleus, while in the zoospores and young thalli they are concerned with the elaboration of oil. Griggs (1909), Percival, Bally, Curtis, and others, on the other hand, claim that they enlarge in the cytoplasm and become the secondary nuclei and primordia of the zoospores. Kusano regards them as reserve food substance which is absorbed in the growth of the resting spore. Whether in *C. replicatum* they are chromidia extruded from the nucleus during division or chondriosomes aggregated around the nuclear membrane has not been determined with certainty. In material killed in ordinary non-chondriosomal fixatives containing acetic acid and stained in haematoxylin and Flemming's triple stain they show a marked affinity for basic dyes and have much the same staining capacity as the nucleole. The same is true when Feulgen's nuclear stain is employed, which supports perhaps the contention that they are of nucleolar origin. However, no marked and characteristic correlative change in the nucleole during the prophases of division such as Kusano, Stevens (1907), Bally, Percival, Curtis, and others have described for *Synchytrium*, have been observed in *C. replicatum*. In the former genus the nucleole is described and figured as periodically extruding globules of chromatin which first accumulate on its periphery and then migrate out into the cytoplasm. As a result the nucleole becomes periodically vacuolated and less basophilic. In chondriosomal fixatives and stains these granules in our species do not appear as sharp, well defined, and specific as chondriosomes. Minute mitochondria may be found in *C. replicatum*, but the larger bodies seem to be distinct from them.

The changes which occur in the nuclear reticulum during the early prophases of division are not very clear and are difficult to interpret correctly. The nucleus increases perceptibly in size, while the chromatin bodies become larger, more basophilic and less highly dispersed. Quite frequently the whole reticulum is more or less withdrawn from the nuclear membrane into a dense and somewhat crescentic or half-moon-shaped network, which appears oriented to some degree on the nucleole as is shown in figures 17 and 18. Such nuclei are strikingly similar in many respects to

those of *Synchytrium* and *Polyphagus* as they have been figured by Guttenberg (1909), Percival (1910), Bally (1911), Tobler (1913), and Wager (1913). Whether this appearance is largely an artifact of fixation in *C. replicatum* or a normal early prophase stage is not altogether certain. In poorly fixed and heavily stained material the chromatin network or reticulum may be highly shrunken and appear as a more or less solid crescentic mass as in figure 17. The degree of aggregation and density varies considerably with the quality of fixation, and it is thus possible to make up a series of stages from that shown in figure 17 to the more normal appearance illustrated in figure 20. Such a series, however, does not seem to constitute a progressive succession of prophase stages, and for this reason I am inclined to regard the contraction as largely an artifact. On the other hand, the orientation of the network on and to some degree around the nucleole is quite common and pronounced during the prophases in the best of preparations and thus seems to be a more normal characteristic. In all such cases the nucleole is separated from the chromatin meshwork by a clear space, which may vary considerably in extent with the quality of fixation.

A network of this type appears to be quite common in *Synchytrium*, *Polyphagus*, and *Olpidium*. In *S. endobioticum*, *S. Anemones*, *S. Puerariae*, *S. fulgens*, *S. anomalum*, and *Olipidium radicale*, Guttenberg, Percival, Tobler, Schwartz and Cook (1928), and Kusano (1930) figure the reticulum as a crescentic and amoeboid body with pseudopod-like extensions connecting it with the nuclear membrane and cytoplasm. Wager (1913, fig. 60) likewise figures it as such in the female gamete nucleus of *P. Euglenae*. They describe it as a condensed network with basophilic chromatic particles lying within its meshes, but do not regard the shape and orientation as essentially characteristic of the prophases. In figure 20 is shown what I consider a fairly early prophase stage in *C. replicatum*, which shows a minimum of contraction from the nuclear membrane. The chromatin granules, angular fragments or bodies, are somewhat larger and denser than in resting nuclei, while the nucleole is rather irregular in outline and denser in certain regions. A somewhat later stage is shown in the nucleus on the left of figure 21. This figure shows a binucleate incipient zoosporangium, but it is interpolated here because both of its nuclei are in early prophase stages of division. The nucleus on the left side contains a number of elongated chromatin strands which doubtless represent an early stage in the development of the spireme. The nucleole is comparatively small, but whether this diminution in size is to be taken as an index that the nucleole has given up chromatin to the spireme is highly uncertain. Quite commonly in more advanced stages after the chromosomes

have been fully formed there appears to have been no reduction in size and staining capacity of the nucleole.

As has been noted before, the majority of cytologists dealing with Chytridiales maintain that the chromatin of the resting nucleus is contained in the large densely basophilic nucleole and extruded into the fine network or nuclear cavity as the prophase stages progress. In *Polyphagus Euglenae, Synchytrium endobioticum, S. Taraxaci, S. Puerariae, S. fulgens, Olipidium radicale*, etc., Wager, Dangeard, Kusano (1907, 1930), Bally, Curtis, Schwartz and Cook, and others describe the chromosomes as being largely derived from the karyosome. In *S. endobioticum*, according to Miss Curtis, the linin strands are attached to the surface of the nucleole, and during the growth of the prosorus three distinct discharges of chromatin from the nucleolus occur. These chromatin globules travel along the strands, and are eventually discharged into the cytoplasm. The prophases of division begin with the third discharge, and Miss Curtis believes that the chromosomes are formed directly from chromatin buds extruded from the nucleole. Kusano found essentially the same type of nucleolar extrusion in *S. fulgens*, but describes the chromosomes as being formed from the nucleolar substance present in the reticulum. In *P. Euglenae* Wager does not show the origin of the chromosomes. Little or no evidence of nucleolar extrusion and the formation of the chromosomes from such material has been found in *C. replicatum*, and I am strongly of the opinion that the nucleole contributes very little if any directly to the chromosomes. The evidence at hand indicates that they arise from the chromatin reticulum entirely. No nucleolar discharges such as those described for *Synchytrium* have been observed, and in the majority of successive prophase stages studied the nucleole has undergone but little change in size and shape.

The nucleus on the right side of figure 21 shows the spireme fully formed. The three strands lying in the plane of focus are definitely oriented on the nucleole and show a zigzag or spiral structure. In their orientation they are strikingly similar to the prophase strands in *S. endobioticum* as they have been figured by Miss Curtis. So far no evidence of longitudinal splitting or doubling has been observed in my material, and it is not yet clear where this process occurs. In figure 21 is also shown the best evidence I have so far found for the presence of centrosomes and astral rays. Deeply staining granules at the poles of the nucleus are not uncommon, but it is usually impossible to differentiate them from the others present or to see any evidence of cytoplasmic radiation in connection with them. In figure 21, however, are shown granules on the membrane of each nucleus with lines radiating from them. On the left-hand nucleus five beaded radiating

strands are present, while on the one to the right only two are visible. Whether or not these granules and cytoplasmic strands are centrosomes and astral rays I am at present uncertain. They are strikingly similar to such structures, but in view of the fact that I have found them so seldom and that they seem to play no visible part in the formation of the spindle and the division of the nucleus and chromosomes I am somewhat doubtful at present as to the presence of centrosomes in *C. replicatum*. Among the Chytridiales which have been studied cytologically centrosomes have been found only in *Synchytrium* amd *Rhizophidium*. Neither Kusano, Wager, Barrett, Němec, nor Schwartz and Cook found them in *Olpidium*, *Olpidiopsis*, and *Polyphagus*. In various species of *Synchytrium*, however, according to Stevens (1907), Griggs, and Kusano, centrosomes and enlarged asters appear in the cytoplasm during the telophasic reconstruction of the daughter nuclei, but never during the prophases, metaphases, and anaphases. Quite often they may appear spontaneously in the cytoplasm quite apart from the daughter groups of chromosomes. In *S. Puerariae* and *S. fulgens* Kusano describes the astral rays as bending around the chromosome groups and fusing to form the daughter nuclear membranes. In view of their activity in this respect he called these structures *karyodermatoplasts*. In *Rhizophidium Beauchampi* Hovasse (1936) figures centrosomes lying at the poles of the nucleus but inside of the nuclear membrane. No astral radiations in the cytoplasm occur in connection with these bodies.

In figure 22 is shown a later prophase stage in which the spireme strands have apparently contracted and are shortening to form the chromosomes. The whole mass is, nevertheless, oriented to some degree on the large disk-shaped nucleole. The nucleus is somewhat lemon-shaped, and at one pole lie two large granules, but they are hardly, it seems to me, to be regarded as centrosomes. A slightly later stage is shown in figure 23. The nucleus appears to be at its maximum diameter, and the nucleole in the shape of a flat ring lies at one side. In the center of the nucleus and apparently imbedded in a light staining karyoplasm lie three elongated and three short discrete bodies which are doubtless the chromosomes. The tip of the longest one lies over the nucleole. While only six bodies are shown in this nucleus I am not certain that this is the correct number of chromosomes for the species. They are usually so small and closely crowded that it is almost impossible to arrive at a definite number. Figure 24 shows eight bodies, while in figures 28 and 29 are shown six and seven, respectively. I am thus inclined at present to put the number of chromosomes in *C. replicatum* between six and nine. The correct number will doubtless be very small and close to those of other chytrids in which the chromosomes have been counted. As far as I am aware, this has been done for only four

genera and seven species, including *C. replicatum*. For the sake of comparison I am presenting these data here in tabular form.

Author	*Species*	*Chromosome number*	
		n	2*n*
Stevens and Stevens (1903)	*Synchytrium decipiens*	4(?)	—
Griggs (1908)	*Synchytrium decipiens*	4	—
Griggs (1909)	*Synchytrium decipiens*	4	—
Kusano (1907)	*Synchytrium Puerariae*	5	—
Kusano (1909)	*Synchytrium Puerariae*	5	—
Kusano (1930)	*Synchytrium fulgens*	5	10
Curtis (1921)	*Synchytrium endobioticum*	5	—
Barrett (1912)	*Olpidiopsis vexans*	6	—
Wager (1913)	*Polyphagus Euglenae*	10–12	—

I have so far been unable to determine the origin of the division spindle. No evidence of polar caps or radial kinoplasm has been found; nor have I seen any entering cones or bundles of fibers in connection with centrosomes such as Harper (1897, 1905) has described in *Erysiphe* and *Phyllactinia*. The source and manner of origin of the spindle in the other chytrids which have been studied cytologically are likewise obscure and little understood. In *Synchytrium decipiens* Stevens and Stevens describe the linin as aggregating near the center of the nucleus, and from which the threads gradually assume the form of a spindle. Kusano (1907, 1909, 1930) is uncertain as to its origin in *S. Puerariae*, but states that it is formed in the center of the achromatic striations. Bally likewise describes it as being formed from the linin in *S. endobioticum*. Curtis, on the other hand, as has been noted above, figures and describes fine linin threads attached to the nucleole in the resting nuclei and prophase stages of *S. endobioticum*, which later on converge at the two ends and thus form the spindle. The number of linin threads is the same as that of the chromosomes, and in most of her figures they look like irregular spireme bands or strands. Her illustrations as to the origin of the spindle are not at all convincing, and I am inclined to agree with Kusano (1930) that her five so-called linin threads are actually fine elongated spireme strands or chromosomes oriented on the nucleole. In *Polyphagus Euglenae* Wager failed to observe the origin of the spindle, while in *Olpidiopsis vexans* Barrett claims that "the chromosomes become massed together into a large, deeply staining body from which the tips of the spindle appear to emerge." The best evidence which I have supporting the *linin origin* of the spindle is illustrated in figure 24. The chromosomes lie more or less in the center of the nucleus, and extending out from them are faint, irregular granular lines, which perhaps constitute the residual

linin. However, I have not observed any further development stages that show the transformation and rearrangement of these lines into the spindle. It is thus quite obvious from this figure and the above description as well as from the survey of the literature that the problem of the source and manner of origin of the spindle in the Chytridiales is still quite obscure. However, there can be no doubt that in the majority of species it is entirely intranuclear until the late anaphases. The only exceptions to this rule occur in the zoosporangia of *Olpidium Brassicae* (Němec, 1912), *O. radicale* (Schwartz and Cook, 1928), and *Cystochytrium radicale* (Cook, 1932), and in the resting spores of *O. Viciae* (Kusano, 1912).

In figure 25 is shown the primary nucleus of a zoosporangium in the late prophases. The intranuclear spindle is completely formed, and the indistinct chromosomes are apparently being drawn into the equator. The nuclear membrane is well defined and clear, and the spindle fills approximately a half of the nuclear cavity. It is comparatively narrow and distinctly spindle-shaped, and its poles are centered on two somewhat disk-shaped bodies lying on the nuclear membrane. From these bodies radiate faint and irregular lines which suggest the presence of astral rays, but here again the evidence is not particularly convincing. Figure 26 shows an equatorial plate stage. In this spindle organ the nucleus has doubtless been distorted during fixation and sectioning, so that the membrane appears abnormal. The division spindle is quite broad in the equatorial region and then tapers rather quickly to the poles, which are again centered on two deeply stained granules. The chromosomes are so closely crowded that they form almost a continuous irregular band across the equator. As a general rule the nucleus becomes somewhat elongated and oval in shape during the late prophases, metaphases, and anaphases as is illustrated in figures 25, 31, 33, and 34, but round nuclei may occasionally be found (figs. 23, 27, 32). In figure 27 the spindle is quite broad and tapers only slightly toward the poles. This figure illustrates very well the arrangement of the chromosomes in a circular band around the outer periphery of the spindle. The nucleole at this stage usually lies at the side of the nucleus and is distinctly crescentic when viewed from the side. In many nuclei it may appear to push the nuclear membrane out or distend it, and in some preparations such as are shown in figures 27, 30, and 32 it may even seem to lie on the outside. This is doubtless an optical illusion, since it is very difficult to distinguish the membrane in that particular region. It is to be particularly noted that the nucleole is still quite large and densely basophilic.

A polar view of the equatorial plate stage is shown in figure 28. Six distinct bodies lie in a flat loop in the center of the nucleus, while the more

or less cup-shaped nucleole occupies the side. Occasionally one pole of the spindle may be centered on the nucleole as is shown in figure 29. The division figure in this cell has doubtless been displaced by the microtome knife in sectioning, which accounts for its unorthodox appearance. This figure shows a 2-celled spindle organ in which one nucleus has migrated to other parts of the thallus, while the remaining one is dividing. So far the actual separation of the individual chromosomes has not been observed, but in figure 30 is shown a very early anaphase stage. The two daughter groups of chromosomes are rather compact and have begun to draw apart, so that a clear space is visible between them. At the upper pole of the spindle lies a flat curved body which stains heavily. The nucleole is very conspicuous, basophilic, and somewhat pyramidal in shape. In over-stained preparations the chromosome groups may look like two irregular but optically homogeneous bands across the spindle as is shown in figure 31, and it is then impossible to differentiate the limits of the individual chromosomes. This figure is particularly noteworthy in that it suggests that the chromosomes separate first in the middle and then progressively towards the periphery. As a result the two groups form a somewhat flat loop with the two ends pulling apart. Figure 32 shows the clearest and best defined case of early anaphase so far seen. The nucleus is quite large and round, while the achromatic spindle is comparatively narrow. The two groups of chromosomes are quite conspicuous, but the individual bodies are still so close together that they form irregular bands. The nuclear membrane is bulged out in the vicinity of the nucleole, and the ends of the latter appear somewhat stretched or drawn out in the direction of the two poles. A later anaphase stage is illustrated in figure 33, in which the individual chromosomes are fairly distinct. Between the two groups run faint central spindle fibers, while the mantle fibers taper quickly at the poles. This nucleus is surrounded by such a dense mass of granules that its membrane is hardly distinguishable, and as a consequence the nuclear region stands out in contrast as a comparatively clear oval space with the division figure clearly defined and sharp. In figure 34 the chromosome groups have reached the poles of the spindle and form two more or less flattened crescentic bands which conform to the boundary of the nucleus at those points. The spindle is quite barrel-shaped in this figure and fills most of the nuclear cavity, which is rather exceptional. This preparation was considerably overstained, which doubtless accounts largely for the density and optical homogeneity of the two chromosome groups. Occasionally in division figures like this there is slight resemblance to some of the so-called promitotic stages which have been described for the Plasmodiophorales, particularly when looking directly down upon the nucleole. This is doubtless due, how-

ever, to poor fixation and overstaining, and so far no evidence has been found as far as the method of nuclear division is concerned that would indicate a possible relationship between this polycentric chytrid and the so-called parasitic slime molds.

Following the arrival of the chromosomes at the poles, the spindle begins to elongate rapidly, and as a result it apparently pushes through the nuclear membrane in these two regions. I have never actually observed this occurrence in *C. replicatum*, but indirect evidence supports this conclusion. The nuclear membrane disappears first at the poles, while in the equatorial region it may persist for some time. In *Polyphagus Euglenae*, according to Wager (1913), the spindle elongates tremendously and pushes through at the poles, while the rest of the nuclear membrane remains intact and becomes compressed like a flattened drum. The same kind of elongation of the spindle has been described for *Synchytrium* by various workers. Figure 35 shows the elongated straight spindle with a remnant of the nuclear membrane on the right side. The chromosome groups are quite compact but not optically homogeneous. The nucleole is here seen in surface view and appears quite large, oval, and densely basophilic. With the breakdown of the nuclear membrane it is liberated into the cytoplasm and persists for a long time. A later anaphase stage is shown in figure 36. The central portion of the cell formerly occupied by the nucleus is comparatively clear, although it has elongated and is being encroached upon by the more dense cytoplasm. No evidence of the nuclear membrane is visible, and the large nucleole lies clear and distinct in the cytoplasm. The spindle is even more elongated and narrow, and consists of but a few fibers connecting the daughter chromosome groups. Furthermore, it is slightly curved and strikingly similar to the spindles in *Olpidiopsis vexans*, the rusts, and other fungi, as figured by Barrett, Blackman (1904), Colley (1918), Dodge and Gaiser (1926), and others. Similarly marked polar radiations in the cytoplasm occur around the groups of chromosomes. Wager (1913) has described and figured such radiations in *P. Euglneae* likewise. A somewhat exceptional case is shown in figure 38. The spindle is still rather broad and well developed, while the nucleole which lies on its periphery is greatly extended and drawn out at the edges. No evidence of the nuclear membrane is visible, and the chromosomes are aggregated into compact optically homogeneous oval bodies. They lie at the bottom respectively of two clear spaces which seem to be partly delimited by thin cytoplasmic membranes or borders. There is thus here a faint suggestion of an early stage in nuclear membrane formation, and for this reason I am inclined to regard this stage as more advanced than the one shown in figure 37, although the spindle is still quite broad.

As to the manner of reorganization of the chromosome groups and the formation of the daughter nuclei there are but few concrete data in the literature and much less agreement of opinion. In *Synchytrium Puerariae* and *S. fulgens*, Kusano describes the chromosome masses as being transformed into a nucleole and then becoming surrounded by a clear space. The astral rays then fold over and fuse and thus progressively build the nuclear membrane around this cavity. Stevens and Griggs' figures on *S. decipiens* support Kusano as to the presence of centrosomes and astral rays during the telophases, but Bally and Curtis failed to find such structures in the cytoplasm of *S. endobioticum* and *S. Taraxaci*. In the latter species Bally reports that the chromosomes unite to form the nucleole, which then becomes surrounded by a clear space and eventually a nuclear membrane. The spindle, however, persists as a fine filament connecting the daughter nuclei long after they have been reconstituted. The so-called nucleole then begins to divide into secondary ones, and very shortly the reticulum appears. Miss Curtis' account for *S. endobioticum* is essentially the same as Bally's. Barrett and Wager contribute nothing to our knowledge of this reorganization from their study of nuclear division in *Olpidiopsis* and *Polyphagus*. As has been noted above I have been unable to add anything of marked significance from my study of *C. replicatum*. The chromosome groups become united into a compact body as is shown in figures 37 to 39, and above and below each group respectively develop clear areas which eventually appear to become surrounded by a membrane. The clear areas and membrane seem to develop and progress gradually from the chromosome masses onward as is shown in figure 37, which suggests at least that they may be directly concerned with the formation of the nuclear membrane. Figure 38 shows the two daughter nuclei completely formed. The chromatin masses constitute two large densely basophilic oval bodies which are similar in every respect to the nucleoli of resting nuclei. No chromatin reticulum is yet visible. The spindle has disappeared entirely, and in its former place is a clear region. The nucleole is still quite large and densely stained and lies in the cytoplasm. However, it usually decreases gradually in size (fig. 39), and may eventually be broken up into a number of small granules (fig. 40) and disappear entirely, as is shown in figure 41. In two instances so far observed it persisted until after cell division had been completed, and as a result was included in one of the daughter cells of the spindle organ.

While the early prophase stages seemed to show that the spireme and chromosomes were formed from the chromatin reticulum rather than the nucleole, the telophase changes suggest the opposite. The evidence at hand indicates that the chromosomes unite to form large nucleole-like bodies

which in some unknown manner become enveloped by a nuclear membrane. At first there is little or no reticulum visible, but as reconstruction continues a faint one develops as is shown in figure 39. Although the nucleole-like chromatin masses show no marked change in staining capacity, shape, or size during the process, this reticulum is undoubtedly formed from it in some fashion. In no cases so far observed have the telophase chromosome masses become alveolated or broken up into smaller and smaller units and gradually dispersed as is the general rule in higher plants; nor have the nucleoli been found to arise *de novo*. The author is at present of the opinion that the nucleole constitutes the residue of the chromosome masses after the reticulum has been formed in the reconstructed daughter nuclei. Very often the space between the two daughter nuclei is highly vacuolated, and occasionally both may move into this region and lie close together as is illustrated in figure 40.

Cytokinesis in the spindle organs and zoosporangia occurs after nuclear division is complete and appears to be entirely independent of the activity of the achromatic spindle. In the former only a few stages have been found to date, so that at present the story is far from complete. As has been noted before, the equatorial region of the cell formerly occupied by the nucleus and spindle is often highly vacuolate, and nothing is to be seen of the fibers by the time cell division begins. In figure 41 is shown the earliest indication of cytokinesis so far observed. The equatorial region of this cell is non-vacuolated, and at the periphery are two faint open furrows which appear to be progressing centripetally. A slightly later stage is shown in figure 42. The furrows are considerably wider and more open at the periphery, and within them have now been formed a thin membrane or cell plate, which is in direct connection with the cell walls and extends almost up to the apex of the furrow. I am still uncertain as to how this membrane is formed at its encroaching tip; no conspicuous, deeply stained cytoplasmic masses or plugs have been found enveloping and preceding it, as Lutman (1911) has shown in *Closterium*. Its development is more similar in superficial appearance at least to the so-called girdle wall formation in *Cladophora* as figured by Brand (1908), although no accumulation of slimy wall substance in the regions where cell division begins has yet been observed. The furrows and cell plate progress deeper and deeper, as is shown in figure 43, until they meet in the center and thus divide the cell (fig. 44). No kinoplasmic material in the form of spindle fibers or astral rays has thus been seen in relation with cytokinesis in the spindle organs. Nuclear division may not always be followed immediately by cell division. In a few instances elongated multinucleate spindle organs, such as are illustrated in figure 45, have been found in which no cross septa were present. Such

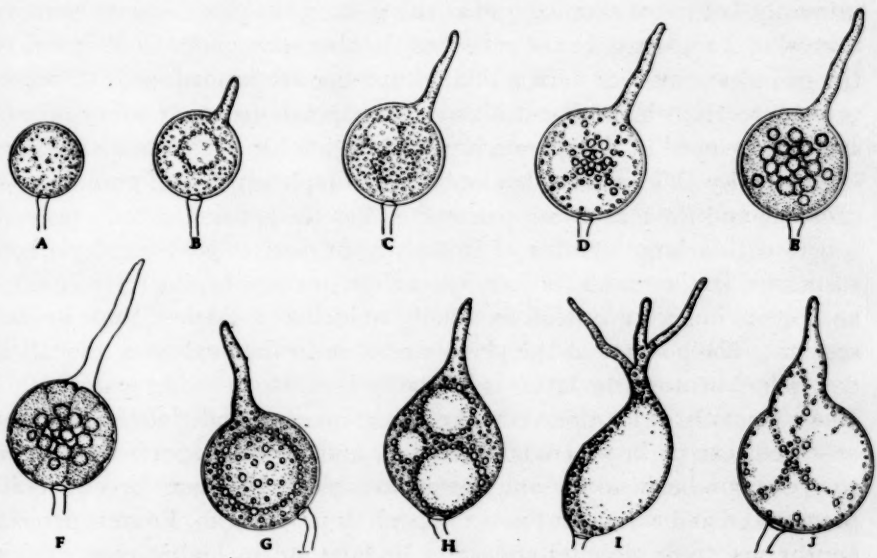
cases, however, appear to be exceptional. Whether cross walls are developed at all in such organs or all of the nuclei migrate out is not known.

As has been shown elsewhere, the spindle organs vary considerably in size, shape, and number of cells. They are predominantly spindle-shaped, but occasionally irregular and somewhat rectangular-shaped ones with several tenuous filaments, such as is illustrated in figure 46, occur. The division spindle in one of these cells is at right angles to the long axis of the organ, and as a result, when cytokinesis is completed the structure may become dictyosporous in appearance. Oftentimes the individual cells may become greatly enlarged and vesicular or transformed directly into zoosporangia. In figure 47 is shown what was doubtless originally a five-celled spindle organ, but two of its cells have developed into sporangia. In exceptional cases the zoosporangia may bud out from the surface of the spindle organ as is shown in figure 48. This figure is of further significance in that it also shows the division of the primary nucleus of the young sporangium.

DEVELOPMENT OF THE ZOOSPORANGIA

The zoosporangia occur terminally and intercalary in the rhizomycelium, and may also arise from the surface of a spindle organ or directly by the transformation of its cells. They vary considerably in size and somewhat in shape, but are predominantly oval or spherical. Occasionally the shape of larger ones is determined by that of the host cell. At maturity they are filled with large, round, refractive, golden-red globules. Their development and maturation can be readily followed in the living condition, and for the sake of comparison with fixed and stained preparations I shall describe the processes first in living material. The most pronounced visible changes involve increase in size of the zoosporangium itself and the genesis of the refractive globules. The visible and physical changes which the latter undergo in the development and maturation of the zoosporangium are quite characteristic and can be followed with readiness. With the view of presenting these changes more concretely, I have drawn in text-figure 1 successive developmental stages of a terminal sporangium at different time intervals. At first these droplets are hyaline or grayish-white in color with a faint tinge of pale orange, and appear to be more or less evenly distributed in the hyaloplasm, as is shown in text-figure 1A. At this stage they vary from minute points at the limits of microscopic visibility to well-defined and fair-sized globules. As the zoosporangium grows in size (text-fig. 1B), they increase in number and size also, and quite often in this uninucleate stage they may be grouped more numerously about the centrally located nucleus. In living material the nucleus cannot, of course, be

clearly seen, but its outline as a hyaline sphere is rather well defined by these globules. Their increased number around the nuclear membrane does not necessarily indicate, in my opinion, a specific affinity between the two, but is doubtless primarily a matter of displacement. Assuming them to be evenly distributed in a more or less viscid hyaloplasm, it is obvious that those in the center around the nucleus will become more closely crowded as it grows in size preparatory to division. This view is supported by observation on the developmental stages of vacuolated zoosporangia,



Text-figure 1, showing changes in the refractive golden-red globules during the maturation of the zoosporangium in living material.

such as are shown in text-figure 1G to 1I. In these sporangia the globules were densely crowded at the periphery of the vacuoles, and as the latter increased in size the crowding became more and more pronounced. Grouping of refractive droplets around the nuclear membrane as described above is not always visible, however. Sometimes it is quite pronounced, but at other times it appears to be lacking. This may possibly be associated with the disappearance of the nuclear membrane in the late anaphases of division and its subsequent reformation in the telophases. It is not inconceivable that the refractive globules may invade the nuclear region after the membrane has broken down; and then as the daughter nuclei are reconstituted and grow in size the crowding again becomes evident.

The increase in number of globules as well as their individual growth continues apace with the growth of the zoosporangium, but when it reaches mature size (text-fig. 1C), there appears to be no further multiplication of droplets. Hand in hand with this growth a marked change in color occurs. As the droplets increase in size the orange tint becomes more and more intense, and by the time the zoosporangium has reached the stage shown in text-figure 1D, the globules are golden red and highly refractive. At this stage (text-fig. 1D) and also earlier, the number of globules begin to decrease in number and increase markedly in size. The latter change is primarily due to coalescence, and as this goes on the globules seem to move toward and aggregate in the center of the zoosporangium. Coalescence of the globules continues until a limited number are formed, as is shown in text-figure 1E, which almost always corresponds to the number of zoospores developed in the sporangium, although a few exceptions have been observed. By this time the hyaloplasm is completely free of granules and droplets, and the mature zoosporangium has the appearance of a pellucid sphere with a large number of brilliantly refractive, golden-red globules suspended in the center. Shortly thereafter cleavage begins (text-fig. 1F), and occurs in such a fashion as usually to include a single globule in each segment. The position of the globule relative to the nucleus is difficult to determine, because the latter is generally invisible in living material.

So far no Brownian movement of these droplets and globules has been observed, except in pathological, dying, and dead zoosporangia. As the zoosporangia begin to die and degenerate, movement may become quite pronounced and active, but never in healthy protoplasm. From superficial appearance these globules appear to be fatty or lipoidal in composition, and they have been almost universally referred to in chytrid literature as oil droplets. Their reaction to various acids and agents, however, indicate a more complex nature. When treated with a 2% osmic acid solution they retain their golden-red color for several hours, and do not blacken conspicuously until two days or more have elapsed. They slowly undergo a change in color from red to reddish green, olive green and finally dark olive. In full strength glacial acetic they change to orange and light olive orange in color, and finally after approximately three-quarters of an hour they become completely hyaline. This change is not accompanied by any marked increase in size or dissolution, and appears to be nothing more than a bleaching process. After 18 hours immersion no further change occurred in these globules. In ether and chloroform the changes were essentially the same. In absolute alcohol immediate coagulation and clumping of the globules took place, and within approximately six hours they had lost all

color and become hyaline without undergoing any perceptible change in size and shape.

The exit tube usually begins to form quite early, and it is not uncommon to find it almost fully developed while a zoosporangium is still uni- or binucleate, as is shown in figures 48, 49 and text-figure 1B. Its length varies from that of a short papilla to a cylindrical tube sometimes 30 μ in length, and it may occasionally branch one to several times (text-fig. 1I).

In fixed and stained preparations these globules as such are no longer visible and appear to have been destroyed or altered markedly during the process of fixation. A young uninucleate incipient zoosporangium is shown in figure 48 in which the nuclear membrane is surrounded by a layer of irregular, densely basophilic granules and fragments of varying sizes and shapes. Do these bodies represent the modified refractive globules of living material, or are they chromidia and chondriosomes? In view of the fact that the refringent globules as such are no longer visible in fixed and stained preparations, and that the angular, irregular and oval granules around the nuclear membrane are not destroyed by fixatives containing acetic acid and are densely basophilic and stain like the nucleole and chromosomes, it seems improbable that they are chondriosomes or the residue of the globules. The nucleus of the zoosporangium shown in figure 48 has doubtless come directly from the upper cell of the spindle organ. The primary nuclei are comparatively large (6 μ to 7.5 μ), but their size in proportion to the secondary nuclei is not even near the magnitude exhibited by *Synchytrium*. They divide mitotically with an intranuclear spindle in the same manner as in the spindle organs. The cytoplasm at this stage is usually quite dense, particularly around the nucleus, and relatively free of large granules at the periphery of the sporangium. Figures 21 and 49 show binucleate stages. In the former there are very few granules in the cytoplasm and none around the nuclei, but in the latter they are unusually abundant and large. They form a broad, dense, and somewhat constricted layer around both nuclei, which have apparently moved closer together after division and now lie in the more or less vacuolated region formerly occupied by the primary nucleus and division spindle. This figure is of further significance in that it also illustrates proliferation of the zoosporangia, which is quite common in *C. replicatum*.

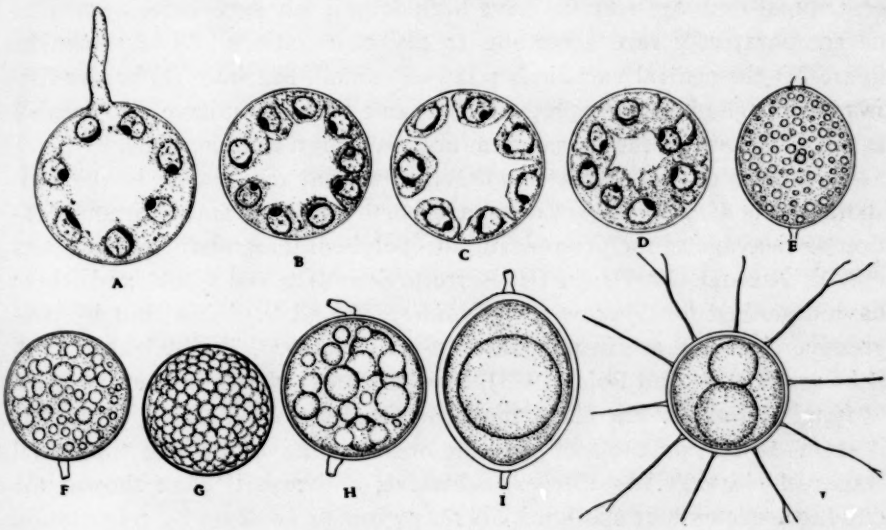
In figure 50 is shown a tetra-nucleate sporangium whose four nuclei are all in the late prophases of division. Definite spireme bands are visible in the two upper ones. Figure 51 shows another zoosporangium of the same stage in which only three of the nuclei are present in the plane of the section. These are markedly globe-like in shape, and are clearly in the equa-

torial plate stage of division with the poles of the intranuclear spindles centered on rather well-defined bodies or granules lying on the nuclear membrane. In two of the nuclei these bodies appear flattened and disk-like in shape. In the third nucleus one end of the spindle is centered on the nucleole. Around none of them, however, are astral or polar radiations visible. Figure 52 shows a portion of an elongated eight-nucleate zoosporangium. Five of the nuclei are clearly visible, while at the base of the figure are faint evidences of two others. Two of the five plainly visible nuclei present profile views of equatorial plate stages, while the remainder show polar views of the same stage. It is thus obvious from these figures that nuclear division in the zoosporangia is distinctly mitotic and completely simultaneous, as is usually the case of zoosporangia in general. Large zoosporangia with sixteen nuclei in the equatorial plate stage of division have been found several times. The number of divisions is directly correlated with the growth and size attained, and as a result the number of nuclei present in mature sporangia varies accordingly. As the number increases the nuclei become gradually smaller, and at the time of cleavage in unusually large sporangia they may be only slightly more than half the size of the primary nucleus. The cytoplasm of the zoosporangia shown in figures 48 to 52 is comparatively homogeneous with no large conspicuous vacuoles. Vacuolated ones, however, are not uncommon, and at maturity the more or less irregularly distributed vacuoles usually flow together to form a larger central one as is shown in figure 53. The cytoplasm in this sporangium is unusually sparse and scant, and the tonoplast of the vacuole is not particularly sharp and well defined. The nuclei of such zoosporangia lie in the peripheral layer of the cytoplasm.

CYTOKINESIS AND ZOOSPOROGENESIS

Cytokinesis in the zoosporangia occurs by the process of progressive cleavage in which furrows cut the protoplasm up into uninucleated segments. It may be centrifugal or centripetal, or in rare cases a combination of both, depending on the size and vacuolation of the sporangium. Figure 54 shows a portion of a 16-nucleated zoosporangium in which cleavage has just begun at the periphery. This preparation is somewhat shrunken, and doubtless as a consequence the furrows are more open than usual. In such a homogeneous sporangium they travel in a more or less radial direction, but may occasionally become curved or bent. In figure 55 is shown a smaller zoosporangium in which the cleavage furrows have progressed almost to the center of the section. In cases where the center is occupied by a nucleus the furrows stop short of it, divide, and the two arms travel almost at right angles to the original direction, as is shown in figure 56.

They then take a more curved path, and as they meet with similar arms of other furrows a more or less round uninucleate segment is delimited in the center. In other cases where the central nucleus is lacking the furrows meet in the center (fig. 57), and as a consequence the cleavage segments are typically wedge-shaped. Figure 58 shows a section of a large zoosporangium in which cleavage is complete, and the segments are primarily pentagonal in shape. The furrows or lines of demarcation are quite marked and clear, and each zoospore initial is surrounded by a faint membrane which seems to be hardly more dense and conspicuous than the remainder of the cytoplasm. In unusually large zoosporangia bi- and trinucleate segments



Text-figure 2, showing progressive cleavage in vacuolated zoosporangia from fixed and stained preparations, and the development of the resting spores in living material.

may occasionally be delimited first, but they are subsequently divided by secondary furrows. Following the stage shown in figure 58 the segments may increase in size and volume and almost completely fill the zoosporangium again. In such instances the lines of demarcation are scarcely distinguishable.

In highly vacuolated zoosporangia cleavage is usually centripetal. Such types are comparatively rare, but for the sake of comparison I have drawn in text-figure 2 successive stages of the process. In figure 2A is shown a median section of a sporangium with a large central vacuole, in which at one side there is some evidence of cleavage furrows. These begin at the tonoplast of the vacuole as rather broad V's and progress outwards between the peripherally disposed nuclei. A slightly later stage is shown in

text-figure 2A. The furrows are quite conspicuous and have deepened considerably. In the zoosporangium illustrated in text-figure 2C, four of the furrows have reached the boundary, while the other three are at the stage of cutting through the plasma membrane. As a result some of the segments are already fully delimited. After cleavage is complete the central vacuole disappears, and the zoospore initials gradually move toward the center of the zoosporangium. This type of centripetal cleavage is exactly similar to what Schwarze (1922), Scherffel (1925), and Couch (1935) have described for *Olpidiopsis*, *Eurychasma*, *Ectrogella*, *Pythiella*, and the Saprolegniales. In two zoosporangia of *C. replicatum* both centripetal and centrifugal cleavage furrows have been found, but such cases appear to be comparatively rare according to my observations. As is shown in figure 2D the central vacuole is relatively small, but from its border furrows are beginning to develop. At the same time, they have also formed at the periphery of the sporangium and are progressing inwards.

It is thus obvious from this description that cytokinesis in the zoosporangia of *C. replicatum* does not occur by a simultaneous fragmentation or cleavage of the protoplasm into polyhedral segments as Dangeard (1890), Kusano (1909), Curtis, Barrett, Schwartz and Cook, and others have described for *Synchytrium*, *Olpidiopsis*, and *Olpidium*, but by progressive cleavage, as Harper (1899), Swingle (1903), Timberlake (1902), Schwarze (1922) and Bold (1933), have so clearly shown in the sporangia of fungi and algae. The claim that it is simultaneous is based, primarily, it seems to me, on the fact that the observers have not seen the initial stages of cleavage. As Harper, Schwarze, and myself have shown, the cleavage segments or spore initials may grow or swell up by rehydration after cleavage is over and often completely fill the sporangium. As a result of this increase in size the lines of demarcation become faint and less conspicuous and look as if they had but recently been formed. The whole sporangium thus appears as if it had at once undergone simultaneous division into polyhedral segments throughout. It is thus apparent that if observations are made at that stage the impression is deceptive.

As has been noted above, the cleavage segments or zoospore initials in sections are usually pentagonal, hexagonal, or triangular in shape. During the late stages of cleavage and the transformation of the segments into zoospores the nucleoli appear to become greatly reduced in size as is shown in figures 57 and 59. This change is not accompanied by budding or fragmentation and nuclear extrusion as far as I have been able to determine. The reduced nucleoli are usually homogeneous in appearance and even in contour, while the cytoplasm may occasionally be comparatively free of basophilic granules (figs. 54–56). At this stage, however,

granules and dark staining substances are usually very abundant, particularly around the nucleus, as is shown in figures 57–59, but whether or not they are of nucleolar origin is uncertain. Oftentimes they may be so dense as to almost obscure a portion of the nuclear membrane. The extranuclear substance or granules shown in these figures are very finely divided, but in the slightly older segments shown in figures 60 to 69 they are very large and coarse and lie around that portion of the nuclear membrane opposite the nucleole. Figure 60 shows five conspicuous bodies or granules of this type. This segment as well as those shown in figures 61 and 62 are from sporangia fixed in Flemming's medium solution and triple stained. When treated thus the granules are usually ruby red in color like the small round nucleole. In figure 61 is shown a later stage of zoosporogenesis in which the number and amount of extranuclear substance and granules have increased markedly. Apparently the bodies fuse as maturation continues (fig. 62), since at maturity their place is occupied by a densely stained, more or less optically homogeneous, somewhat hemispherical cap which envelops the upper portion of the nucleus, as Kniep (1929) and Hatch have recently described in *Allomyces*. At this stage the zoosporangium with its zoospore segments may frequently have the appearance shown in figure 63. The nuclei may look like clear spaces or vacuoles surrounded at the apex by a densely stained crescentic mass or cap.

Nuclear caps of this type seem to be fairly common in the zoospores of the Oomycetes, according to the observations of Thaxter (1896), Barrett (1912b), Kniep, Cotner (1930) and Hatch; but with the exception of Debaisieux's (1920) account of *Coelomycidium* this description of their presence in *C. replicatum* is the first conclusive report for the Chytridiales. I have also found them in the zoospores of *Catenaria* and *Diplophlyctis*. The figures of Hovasse (1936) suggest that they may be present in *Rhizophidium*, and it is not improbable that the crescentic shaped body or nucleole described by Wager as lying inside of the nuclear membrane of *Polyphagus Euglenae* may possibly be a nuclear cap.

There is, however, considerable difference of opinion as to whether the nuclear caps are intra- or extra-nuclear. Thaxter, Cotner and Sparrow (1933b) interpreted the densely basophilic crescentic bodies which they observed as constituting the greater part of the nuclei, while Barrett described them as food bodies in which the nucleus is partially imbedded. Kniep and Hatch claim that they are entirely extramatrical and fit as a more or less hemispherical hood over the nucleus of the zoospores and gametes. My observations on *C. replicatum* confirm their account as to its spatial relation to the nucleus, but differs somewhat from that of

Debaisieux and Hatch as to its composition. After cleavage has been completed in *Coelomycidium Simulii*, according to Debaisieux, and the segments have begun to round up large angular, round, or elongated basophilic bodies appear in the cytoplasm and gradually fuse into a large body, which usual lies at the apex of the nucleus on the side opposite the point of attachment of the cilium. He regards this extranuclear body as an accessory nucleus which results from the fusion of chondriosomal granules and is in some way connected with the formation and development of the cilium. In *Olipidium*-like parasites of *Saprolegnia* Varitchak (1931) describes and figures the chondriosomes as aggregating around the nuclei during zoosporogenesis, but he does not show their fusion to form an extra-nuclear cap. Hatch has subsequently presented a viewpoint somewhat similar to that of Debaisieux as to the origin of the cap, although apparently unaware of the former's account. He describes the extranuclear cap as originating by the aggregation, vesiculation and fusion of chondriosomes around the nuclear membrane during gametogenesis in *Allomyces arbuscula*, and believes it is equivalent to the so-called limosphere which appears during spermatogenesis in certain mosses. Figures 27, 28, and 32 of Hatch are fundamentally similar to my figures 60, 61, 62 and 63 of *C. replicatum*, but in the latter these extranuclear granules which eventually form the cap show much the same reaction, staining capacity and affinity as the nucleole and chromatin. It is to be noted in this connection that Debaisieux also demonstrated their presence without the aid of chondriosomal fixatives and that they appeared densely basophilic when ordinary killing agents and stains were employed. In *C. replicatum*, as has been noted previously, I have so far been unable to demonstrate with conclusive certainty and to my own satisfaction that they are chondriosomal in appearance and composition. It is to be noted, however, that in *A. arbuscula*, according to Hatch, the chondriosomes are conspicuous in the living hyphae and young zoosporangia as elongated rods, and such material is apparently more favorable for study than *C. replicatum*. Furthermore, the use of Feulgen's nuclear stain has not differentiated the cap specifically in our species. In the best of preparations it has not appeared as a colorless region in contrast to the densely stained chromatin, as Hatch has shown in figure 20 of *A. arbuscula*. The author thus feels that before any definite conclusion can be reached as to the composition of the nuclear cap an intensive study of its occurrence in a large number of species must be made. In *Rhizophidium Beauchampi*, for instance, Hovasse (fig. 4C) figures a densely stained mass at the anterior end of the cleavage segment which in living material blackens intensely with osmic acid, and appears later in zoospores as an oil globule where it

is ultimately used up as food. Whether or not this mass is equivalent to the nuclear cap is uncertain, but its presence, nevertheless, emphasizes the need for more intensive study of the cytoplasmic constituents of the sporangia and zoospores of the lower fungi.

In some zoospores the cap may be reduced and small in size, while in others it may envelop the large part of the nucleus, as is shown in figures 64A and 64B. The nucleole usually lies at the opposite pole of the nucleus, which by this time has become somewhat elongated and even peaked, and is quite small in comparison with its former size. As the zoospore initials mature and begin to glide about in the zoosporangium, the cilia are formed. These are apparently developed before the zoospores escape, since numerous ones have been found with well-developed organs of locomotion while still within the sporangium. Figure 65 shows the structure of the zoospore after it has escaped. The cilium is posteriorly attached, and from its point of insertion runs a faint cytoplasm strand to the nucleole. This strand is not always clearly visible, but it is doubtless equivalent to the rhizoplasts which occur in the zoospores of the Myxomycetes, water molds, and protozoa.

DEVELOPMENT OF THE RESTING SPORANGIA

The resting sporangia of *C. replicatum* are predominantly spherical in shape, and vary from 9 to 21 μ in diameter, but oval, lemon-shaped and elongated ones are not uncommon. They are hyaline and possess a fairly thick wall which is often quite smooth, but in a large number of sporangia fine hyaline, single, and sometimes branched filaments or spines radiate from the surface, as has been described previously by Sparrow (1933) and myself (1934). As many as thirty such threads varying from 4 to 15 μ in length have been found in a single sporangium. It thus seems that both smooth and spiny resting spores are formed in this species. In living material a large spherical, hyaline, and somewhat refractive globule occupies the center of the cell, and in elongated sporangia two or more such globules may be present.

The resting sporangia may be borne terminal or intercalary on the rhizomycelium, and generally make their appearance as the thallus matures. So far no fusion of motile isogametes such as occurs in *Olpidium* and *Synchytrium* have been observed in relation to the genesis of these spores. This possibility, however, must not be excluded, in spite of the evidence at hand that their early development is fundamentally similar to that of the zoosporangia. They begin as enlargements in the rhizomycelium, and in their early stages are indistinguishable from the ordinary evanescent zoosporangia. Very shortly, however, it becomes obvious that the sus-

pended globules in the cytoplasm do not acquire the brilliantly golden red color as in the case of the zoosporangia, but remain hyaline or faint yellow. This seems to be the first recognizable difference. Text-figure 2E shows an early developmental stage in which the globules are still quite small. In text-figure 2F they have begun to coalesce into larger bodies, and their number has been somewhat reduced. The wall has also thickened considerably. It is not uncommon, however, to find young spores which are completely filled with hyaline bodies of more or less the same size as is shown in text-figure 2G. A fairly late stage is shown in text-figure 2H in which the wall is markedly thicker. The hyaline globules are further reduced in number but much larger in size. Eventually they coalesce to form one or two large central globules as is illustrated in text-figure 2I. This figure shows a mature, thick, smooth-walled spore, while text-figure 2J shows one with the slender radiating filaments or spines. So far I have not followed the development of the latter type and determined the manner of origin of the spines. Occasionally the central globule may have a faintly yellow or orange tinge.

In fixed and stained material, on the other hand, the appearance of the developing spores is somewhat different. The globules are apparently destroyed or greatly modified as such by fixation and their places appear to be occupied by vacuoles. Here it becomes apparent that the spores attain mature size without nuclear multiplication ordinarily, and the first visible change is in the thickening of the wall. In figure 66 is shown an incipient uninucleate spore in which the wall is only slightly heavier than in the zoosporangia. The cytoplasm is relatively dense, particularly around the nuclear membrane. The nucleus is large and round with a well-defined nucleole, but the chromatin reticulum appears denser and more basophilic. As the spore matures this change becomes more conspicuous, and the nucleus may be filled with large irregular granules. A later stage in development is shown in figure 67. The wall has thickened perceptibly, large vacuoles have appeared in the cytoplasm, and the nucleus has been slightly displaced from its central position. Furthermore, it is enveloped in a rather dense layer of cytoplasm in which granules are beginning to appear. While the resting spores are predominantly uninucleate, occasional bi- and tetranucleate ones may be found as is shown in figures 68, 69, and 70. Figure 68 shows a binucleate spore in which the wall is comparatively thick. Most of the cytoplasm is aggregated around the two nuclei and is filled with densely stained granules. As a result, the nuclei in such cells appear as relatively clear spaces in which lie the nucleole and other large dense chromatic bodies. In the nuclei shown in figure 68 the chromatin reticulum is condensed into a more or less solid homogeneous body.

I do not interpret this binucleate condition as delayed karyogamy following cell fusion, as Kusano, Griggs, Wager, Schwartz and Cook, and Sampson (1932) claim in *Olpidium*, *Monochytrium*, and *Polyphagus*. It seems to me we are here dealing with an incipient binucleate, arrested zoosporangium which encysted and became a resting spore. This view is supported by evidence of the type presented in figures 69 and 72. In figure 69 a partly developed but unopened sporangial neck is present, which was doubtless formed in the early stages of development and would have served as the exit tube of a zoosporangium. Instead of developing into such a structure, however, its content retracted somewhat, encysted, and formed a thick wall. Here again conspicuous vacuoles are present, and the denser cytoplasm is aggregated around and between the two nuclei. Figure 72 shows a uninucleate but somewhat similar cell in which the exit tube is longer and the spore wall thicker. These figures are fundamentally similar to those of Buckley and Clapham (1929) for *Catenaria anguillulae*, in which they show that the resting sporangia are nothing but encysted zoosporangia. Occasional tetranucleate spores may also be found, as is shown in figure 70. Such sporangia are hardly to be regarded as stages in germination, since the wall is not fully developed. Without doubt, this represents again an incipient four-nucleate zoosporangium which has encysted.

Quite often the spindle organs themselves may develop thick walls and become transformed into resting sporangia, as is shown in figure 71. This is additional indirect evidence, it seems to me, that no sexual fusion is involved in the formation of the resting spores. In figures 73 and 74 are shown fully mature dormant sporangia. The nucleus generally lies flattened at one side (fig. 74) in the primordial utricle and is surrounded by a dense layer of cytoplasm in which a large number of granules are suspended. The large refractive globule which is usually so conspicuous in living material has apparently been destroyed or greatly modified in fixation, since it is no longer present as such. Its position is usually occupied by a large central vacuole. Figure 73 shows the same kind of spore as seen from above. The flattened nucleus here appears quite large and round, with a conspicuous nucleole at one side and a large angular densely stained body lying in the reticulum. The granules in the cytoplasm are very numerous and form a dense layer around the nucleus. At this stage the resting sporangium may be described as mature. So far no germination stages have been found in fixed and stained material.

II. CYTOLOGY OF THE CHYTRIDIALES IN GENERAL

SYNCHYTRIACEAE

The species of this family were the first to receive serious attention from cytologists and have without doubt been most extensively and intensively studied. It is primarily for this reason that I take this family first for consideration. At present it is generally regarded as including only three genera, *Synchytrium*, *Micromyces* and *Micromycopsis*.

In 1890 Dangeard described and figured the nuclei of *Synchytrium Taraxaci* as dividing both by successive bipartition, or amitotically, and indirectly, and from his description and drawings it appears as if mitosis and amitosis occur simultaneously and side by side in the same thallus. Three years later in the same species Rosen (1893) reported direct division of the primary nucleus, in which the chromatin spins out into a spireme, the nucleole divides, and its two halves then migrate apart. Finally the nucleus with its spireme and two nucleolar portions constricts and divides into two daughter nuclei without the formation of an achromatic spindle. Subsequently, the successive divisions of the secondary nuclei assume more and more the character of mitosis, and eventually divide entirely by this method. Thus, according to Rosen, mitosis gradually evolves from amitosis in the growth and maturation of the sorus.

As to the mode of cell division Dangeard described the prosorus of *S. Taraxaci* as fragmenting simultaneously into a large number of polyhedric segments which subsequently form thin membranes, separate, and develop into sporangia. In 1899 Harper, however, demonstrated the inaccuracy of Dangeard's description, and showed in *S. Taraxaci* and *S. decipiens* that cell division is accomplished by progressive cleavage, rather than by simultaneous fragmentation. Cleavage furrows begin at the surface and progress deeper and deeper in a more or less radial direction until they intersect and thus divide the protoplasmic mass into irregular segments. At first the furrows or grooves are so narrow as to appear as thin plates, or lines; but as the result of shrinkage which usually accompanies cleavage the segments begin to separate, and the cleavage lines become open furrows. After cleavage has been completed the prosorus swells to its original proportions, and the segments thus become closely pressed together. The lines of demarcation are nevertheless evident, but at this stage the whole prosorus looks as if it had undergone simultaneous cleavage or fragmentation. From this description it is evident that what Dangeard and subsequent workers saw was only the rehydrated stage of the prosorus after cleavage had been completed, and this doubt-

less explains the oft-repeated description in the literature that cell division in the zoosporangia of the chytrids occurs by simultaneous cleavage.

The problem of the method of nuclear division remained as Dangeard and Rosen had described it until 1903, when Stevens and Stevens undertook a study of karyokinesis in *S. decipiens*. They described the primary nucleus as decreasing tremendously in size preparatory to division, but none the less dividing mitotically with the formation of a definite spireme, chromosomes, and an intranuclear spindle. Lowenthal made a cytological study of *S. Anemone* in 1905, showed the hypertrophy of the infected host cell, and figured the primary nucleus of the resting spore as continuous with the cytoplasm and totally devoid of a membrane. Beyond this peculiarity, which is undoubtedly an artifact, he contributed nothing of significance to our knowledge of nuclear and cell division. Two years later Rytz (1907) published an extensive account of the host range of species of *Synchytrium* and dealt in some detail with the cytology of *S. Succiase*, in which he confirms Harper's description of progressive cleavage in detail. Although he figures a typical mitotic spindle for one of the secondary nuclei, he is, nevertheless, somewhat doubtful as to the universality of this method of nuclear division. Kusano (1907–1908) in *S. Puerariae* confirmed the account of Stevens and Stevens as to the indirect division of primary nucleus, and in the same year F. L. Stevens (1907) independently gave another account of the nuclei and their method of division in *S. decipiens*, *S. fulgens*, and *S. papillatum*. In these species he figured and described not only nuclei without limiting membranes, but centrosomes, astral rays, and mitotic division of some secondary nuclei. He was unable to find intermediate and progressive division stages in the latter, and because of this fact he postulated that a single nucleus may often fragment simultaneously into several smaller ones. Stevens apparently turned over his material of *S. decipiens* to Griggs, who in 1908 described in detail the relation of the centrosome and astral rays to the reconstruction of the nucleus. In the following year (1909a), however, Griggs confirmed Stevens' hypothesis as to amitosis or fragmentation of the secondary nuclei and described two types of nuclear origin, heteroschizis and nuclear gemmation. In the latter process the karyosome breaks up into bodies which then migrate through the nuclear membrane, becomes enveloped in a vacuole, and then becomes surrounded by a membrane. In heteroschizis, on the other hand, the nucleus undergoes direct multiple division or fragmentation to form a somewhat compact morula-like cluster of daughter nuclei. These remain close together, and eventually divide mitotically to form a group of small division spindles. Subsequently in the same year Griggs published another paper (1909b) dealing with these latter mitotic

divisions and the individuality of the chromosomes. From the fact that nuclei derived by amitosis also show the characteristic four chromosomes he concluded that there is no continuity of these bodies from one nuclear generation to another, and "that the chromosome number is a physiological constant, like the other hereditary characters of the species."

In the meantime, Kusano (1909) published a long account of the cytology of *S. Puerariae* and showed again that the division of the primary and secondary nuclei is distinctly mitotic, although he did not find all of the successive stages of the process and also gave some figures of small nuclei which are strikingly similar to those of Griggs. Cell division in the prosorus occurs by simultaneous cleavage, and the sporangial walls are precipitated from the cytoplasm. It seems obvious, however, from his description and figures that he is describing the rehydrated stage of the prosorus which Harper found to occur after cleavage has been completed. At the same time Guttenberg (1909) described and figured a peculiar looking mitotic figure in *S. anomalum*. The nucleus appears to be surrounded by an extraordinarily thick membrane or wall and contains a number of nucleolar fragments. Its upper end, however, is broken open and through this large aperture extrudes the greater part of a division figure apparently in late anaphase. At the exterior pole of the latter lies a large centrosphere. From the appearance of this one figure Guttenberg, nonetheless, concluded that nuclear division in *S. anomalum* is mitotic. In the following year (1910) appeared Percival's study on the life history and cytology of the potato wart disease organism which he transferred to the genus *Synchytrium* under the name *S. endobioticum*. He gave a brief but rather well-illustrated account of the development of the summer sorus, figured what appears to be early prophase changes of primary nucleus, but failed to find metaphase, anaphase, and telophase division stages. According to his description, the primary nucleus begins to shrink as chromatin is extruded into the cytoplasm and gradually disappears altogether. The extruded chromatin granules or chromidia in the cytoplasm then become surrounded by vacuoles and develop into small secondary nuclei, which subsequently undergo mitotic division. This is essentially like the process of nuclear gemmation described by Griggs. In view of the fact presumably that he could not find any sharply defined division figures of the primary nucleus, Percival came to the same conclusion as Dangeard and Rosen that a gradual evolution of typical mitosis occurs in the later divisions of the secondary nuclei. On the other hand, he confirmed Harper as to cell division by progressive cleavage in the prosorus. With respect to the maturation of the thick-walled winter spores or sporangia his account is essentially the same and as curious as for the

prosorus, particularly in relation to the nuclei. The primary nucleus lacks a membrane until it reaches maximum size. Chromatin is then apparently extruded into the meshes of the cytoplasm and eventually gives rise to the nuclei of the zoospores. While this is going on the primary nucleus shrinks in size, the nuclear membrane dissolves and disappears, and at the times the zoospores are formed only a residue consisting of the remnants of the nucleole and chromatin remains.

Percival's work was shortly followed by that of Bally in 1911 on *S. Taraxaci* and *S. endobioticum*. In the former species Bally failed to find any division of the primary nucleus and reported not only nuclear gemmation of the type described by Griggs for *S. decipiens* but also the presence of macro- and micronuclei, and fusion of the small secondary nuclei as well. Later on such nuclei in the sorus and sporangia were found to undergo typical simultaneous mitosis. Cell division was found to occur fundamentally by progressive cleavage, although he does not give any convincing figures of the process. For *S. endobioticum* Bally confirmed Percival's account of the nuclei in detail, but failed to find any division figures of the secondary nuclei. Two years later Tobler (1913) monographed the genus *Synchytrium*, and while her study was largely morphological and taxonomic, she none the less reviews the cytology of the group and in *S. aurantiacum*, confirms Lowenthal's, Stevens', and Percival's description of primary nuclei without membranes. In *S. mercurialis* as well as in *S. aurantiacum* she described and figured migration of secondary nucleoli through the nuclear membrane into the cytoplasm where they develop into small secondary nuclei.

Rytz resumed his study on the cytology of *S. Taraxaci* in 1916 and 1917 with an extensive description and illustration of young sori and micro- and macronuclei similar to those figured by Stevens (1909), Griggs, and Bally. He described these size differences as possibly due either to non-synchronous nuclear division or reunion of recently divided nuclei, and refuted emphatically the views of Griggs, Percival, and Bally that secondary nucleoli normally migrate through the nuclear membrane and develop into nuclei in the cytoplasm. Such phenomena are nothing but abnormal and pathological appearances induced by the fixing agents employed. Likewise, he denied the claim of Dangeard, Rosen, Griggs, Percival, and others that amitosis and mitosis of the secondary nuclei alternate during the growth of the prosorus. He found numerous nuclei which appeared to be dividing directly, but attributed this entirely to artifact induced by the killing solution. Although he did not observe division of the primary nucleus, Rytz, nevertheless, maintained that mitosis is the only method of division of both the primary and secondary nuclei. In 1919,

however, Bally refuted these claims of Rytz, and reiterated his previous views as to chromidial extrusion, methods of nuclear division and zoosporogenesis. In answer to the contention that regular mitosis is essential to equal and proper distribution of hereditary characters he claimed that these were very few in *Synchytrium*, and that the small number of genes which determined them required no elaborate process like meiosis and mitosis for their segregation and distribution. Accordingly, he regarded the chromidia which are extruded from the primary nucleus of the zygote or resting spore as the bearers of hereditary factors.

Following Rytz's and Bally's publications came Miss Curtis' extensive cytological study of *S. endobioticum* in 1921. As to the prophasic changes in the primary nucleus, she described three periodic discharges of chromatin from the nucleolus into the cytoplasm during the growth of the prosorus. According to her report the nucleus then divides mitotically and is followed by similar divisions of the daughter and successive secondary nuclei. Curtis, nevertheless, figures large and small secondary nuclei in the sorus like those described by previous workers. Cleavage in both the prosorus and sorus is described as simultaneous instead of progressive. Perhaps the most outstanding contribution by Curtis was the demonstration of the relationship between the fused gametes and the resting spore. Copulation of motile cells had been first figured in *S. Taraxaci* by Lowenthal in 1905a, but its significance had not been fully recognized. Curtis figured abundant fusion in *S. endobioticum* and claimed that the zygote develops into the resting spore. Her account of the development of the latter and the zoospores, however, is very unusual, and confirms in essential details that of Percival. In the early stage of host infection and growth of the zygote, the nucleus is "represented by a single chromatic globule lying free in the mass of the cytoplasm," which becomes surrounded by a vacuole and develops into a normal nucleus. As the latter assumes mature proportions the nucleole periodically discharges chromatin into the nuclear cavity and cytoplasm. Following the final discharge the primary nucleus shrinks in size and finally becomes functionless without undergoing division. The extruded chromatin granules in the cytoplasm grow somewhat in size and become the primordia of the zoospores, discharging, in the meantime, smaller granules of their own into the surrounding cytoplasm. The zoospores are thus not formed at all by cleavage of the sporangium into uninucleate segments, but develop and enlarge around extruded chromatin granules. The latter, as far as can be made out from Miss Curtis' ambiguous description, eventually enlarge themselves and become the nuclei of the zoospores.

In the same year, on the other hand, Welsford (1921) described and

figured normal intranuclear mitotic figures of the secondary nuclei in the resting sporangium of the same species. However, there has been some doubt expressed (Kusano, 1930) as to whether he was dealing with the resting spore. The results of Miss Curtis as to the degeneration of the primary nucleus and the origin of the secondary nuclei and zoospores were fully confirmed by Köhler in 1923 for *S. endobioticum* and *S. anomalum*, and in 1926 Quintanilha reported and figured nucleolar extrusions and amitosis of the secondary nuclei in the sorus of *S. papillatum*, which were then followed by regular mitosis.

The cytology of *Synchytrium* remained thus until Kusano (1930) published his account of nuclear and cell division in *S. fulgens*. He found not only indirect division of the primary and secondary nuclei of the sorus but in the resting spore as well. Fusion of gametes occurred abundantly to form diploid zygotes. Accordingly, the first division of the primary resting spore nucleus proved to be heterotypic and the subsequent ones homoeotypic. Abundant extrusion of chromatin into the cytoplasm occurred, but instead of the granules becoming secondary nuclei and primordia of zoospores, as most of the earlier workers had claimed, he found them enlarging to form reserve food globules, which are consumed in the germination of the resting spore. As to the method of cell division, Kusano is somewhat obscure. He merely states that the sorus undergoes segmentation by "the formation of the plasmic walls between the segments." Nothing is known apparently concerning the delimitation of zoospores during germination of the resting spore.

Nothing of significance has appeared in the literature on the cytology of *Synchytrium* since Kusano's latest contribution. In a neighboring genus, *Micromyces*, however, Couch (1932) has shown the developmental stages and the host-parasite relationship of *M. Zygogonii* in *Spirogyra*. As soon as the parasite enters the host cell it moves toward the nucleus, and throughout its whole development usually lies closely appressed to the latter. The content of the prosorus escapes into a vesicle, nuclear division, and cleavage into zoosporangia follow. The sporangia then cleave into minute uniciliate zoospores, which Couch claims are planogametes and fuse to form diploid biciliate zygotes. These infect the host cell, but Couch has not so far observed their development into resting spores. No details as to the mode of nuclear division and type of cleavage are given. The genus *Micromycopsis* was established and described by Scherffel in 1926 and confirmed by Sparrow and by Cejp in 1932 and 1933. Nothing is known concerning nuclear division, cytokinesis, and sexuality in this genus.

It is quite obvious from this brief résumé of the literature that there

is little agreement on the manner of nuclear and cell division in the family Synchytriaceae. All but three of the investigators who studied these problems describe the nuclei as dividing either amitotically in one stage of development or mitotically in another, and there is only one detailed and accurate account of cytokinesis for the whole group. The descriptions and views of Griggs, Percival, Bally, and Curtis on nuclear and zoospore development in various species of *Synchytrium* are fundamentally hardly more than a revival in slightly modified form of Schleiden's (1838) and Schwann's (1839) archaic account of "free cell formation" around a "cytoblast," which proved to be such an enigma and stumbling block to a correct interpretation of the manner of origin of new cells. According to these students of *Synchytrium*, neither meiosis nor mitosis occurs in the zygotic primary nucleus of the resting spore; and the secondary nuclei arise from extruded granules in the cytoplasm around which are subsequently developed the zoospores. We see further in these accounts a return in essence to the views of R. Hertwig, Schaudinn, Dobell, Schaxel and other students of the protozoa on the extrusion of chromatin or chromidia from the nucleus in *Arcella*, *Arachnula*, *Actinosphaerium*, etc., and their subsequent union and growth to form the nuclei of the daughter cells. These observations were the basis for the elaborated chromidial hypothesis of Hertwig, Goldschmidt, and others as to the duality of the nucleus, which has been greatly discredited by the development of chondriosomal and Golgi body fixatives and the demonstration of such structures in the cell. If the observation of Griggs, Percival, Bally, Curtis, Köhler, and others on nuclear formation in *Synchytrium* are correct what becomes then of the universal doctrine of *omnis nucleus e nucleo* and its concomitant relations to the present-day concepts of heredity?

As to sexuality in this family fusion of motile isogametes has been described in three species of *Synchytrium* and one of *Micromyces*. In all cases reported the gametes are formed in the same sporangia and there seems to be no genotypic sex differentiation and segregation. Köhler (1930, 1932) has found in *S. endobioticum* that fusion may occur between three or more gametes with the result that the zygotes may become tri- and tetranucleate. He is uncertain as to whether or not all of the nuclei fuse and has not followed the development of such zygotes beyond the early stages, so that nothing is known as to their chromosome number. In *S. fulgens* Kusano regards the sexuality exhibited as relative rather than fixed and absolute, and thus brings this species in line with what Hartmann (1925) and Jollos (1926) have claimed for species of *Ectocarpus* and *Dasycladus*. The gametes all behave as male in the active swimming stage and as female after becoming quiescent. Each gamete is

furthermore unstable in sex character and may act as female in relation to one gamete and as male toward another. Köhler (1936) has reported the same type of sexuality in *S. endobioticum*.

The majority of workers on this chytrid family are more or less agreed on the mass effects of the host-parasite relationship, particularly in *Synchytrium*, but differ somewhat as to the cytological changes incurred. The presence of the parasite leads very shortly to a marked increase in size of the infected host cells and does not inhibit but may actually stimulate neighboring cells to divide also, if the latter are young and meristematic. Lowenthal, however, denies this in *S. Anemones*, but Bally, Curtis, Tobler, and Kusano have shown in *S. endobioticum* and other species that the infected host cells together with the healthy ones surrounding them may divide a number of times, and as a consequence the resting spores often become deeply imbedded in the tissues. Galls or enlargements are accordingly formed in the infected regions, which are thus the result of both auxesis (cell enlargement) and meresis (cell multiplication). Auxesis, however, seems to be the dominant factor in their development. In *S. Puerariae* Kusano (1907, 1909) maintains that the epidermal cells are never attacked, and the zoospores infect only the non-chlorophyll bearing parenchyma by entering the intercellular spaces beneath the stomata. After entering the host cell, the parasite stimulates it to enlarge slightly, but the growth of the *Synchytrium* thallus is proportionally much greater. As a result the parasite soon completely fills it, and the host nucleus and cytoplasm form a thin enveloping layer. With continued growth of the parasite the adjacent host cells are compressed, and an enzyme presumably is then secreted by the former which dissolves the cell walls. The cytoplasm and nuclei of these surrounding cells flow out and unite with that of the initial infected cell to form a dense living protoplasmic layer, which Kusano calls the "symplast." According to this description the cytoplasm and nuclei of the affected host cells are only slightly deformed and enlarged in size and volume by the parasite and remain alive and normal until after zoosporogenesis. The cells of *Oenothera sinuata*, however, fail to enlarge to any appreciable extent when infected with *S. fulgens*, which is primarily due to the relative resistance of the host. The cell is, nevertheless, killed and its nucleus and cytoplasm degenerate simultaneously with the parasite.

In other species, however, the reaction between host and parasite is quite different, according to the accounts in the literature. In *S. Anemones*, *S. mercurialis*, *S. Succisae*, *S. endobioticum*, etc., Lowenthal, Rytz, Guttenberg, Percival, Tobler, and others describe and figure the host nucleus as becoming greatly enlarged and irregular and finally disintegrating.

Guttenberg and Tobler in particular describe host nuclei which have attained a diameter of more than 50 μ and are quite irregular in outline and extensively ramified by furrows and canals. Such changes are usually accompanied by a marked increase in size of the nucleole and its subsequent fragmentation and division into a great number of secondary nucleoli and granules, which then enlarge and become intensely basophilic. Similar but less marked changes may also occur in the neighboring uninfected cells. In some species the host cytoplasm is described as more dense and alveolar than normal, but as the cell enlarges it becomes highly vacuolated. The figures in the literature thus indicate that parasitism may be accompanied by some protoplasmic accretion, but vacuolation without doubt plays the significant rôle in subsequent enlargement or auxesis.

All species of *Micromyces* so far described cause marked local hypertrophy of the infected cells but do not stimulate them or adjacent ones to divide. The chloroplasts soon change to light yellow and brown and together with the rest of the cytoplasm soon become disorganized, degenerate and clumped about the prosorus. *Micromycopsis*, on the other hand, is not particularly destructive, according to Scherffel (1926) and Cejp (1932, 1933) and does not cause any enlargement of the parasitized cells.

WORONINACEAE

This family is a heterogeneous collection of species with biciliate zoospores, and in the present state of our knowledge should perhaps be regarded more as a dumping ground for the *Olpidium*-like forms with swarm cells of this type rather than a group of phylogenetically related species. In recent years many genera and species of the Olpidiaceae have been transferred to this family with the discovery that the zoospores are biciliate. In several species the swarm cells are diplanetic and somewhat reniform in shape with the cilia laterally attached, while sexual reproduction is distinctly heterogamous; and for these reasons they are regarded by many mycologists as distantly related to or the direct ancestors of the Saprolegniales.

It was in a genus of this family that sexuality was first demonstrated for the Chytridiales. In 1872 Cornu showed that the resting spore of *Olpidiopsis* is formed by the fusion of the contents of two unequal thalli. Pringsheim had observed and figured the male thallus attached to the resting spore as early as 1860 and assumed that some type of sexual fusion had occurred, but he, none the less, mistook the zoosporangia for the antheridia of *Saprolegnia*. He showed the conspicuous hypertrophy of the

host cells, followed the development of the zoosporangia in this form as well as in what we now recognize as *Rozella* and *Woronina*, and described zoospore formation as occurring directly (*unmittelbar*). Cornu likewise describes it as such. In 1878 Reinsch observed for the first time actual fertilization and fusion of the male and female thalli. However, in 1880 and 1882 Fischer denied Cornu's and Reinsch's claims as to sexual reproduction in *Olpidiopsis* and described cytokinesis in the zoosporangia as a breaking up of the protoplasm into polyhedral masses. In 1890 Dangeard likewise failed to observe fusion and described cytokinesis as a grouping of the protoplasm around each nucleus. His figures, however, certainly suggest cell division by progressive cleavage. Ten years later Fischer realized his confusion, segregated all *Olpidiopsis*-like species in which sexuality had not been observed in a new genus *Pseudolpidium*, and confirmed the accounts of Cornu and Reinsch. Since that time sexual reproduction in various species of *Olpidiopsis* has been observed again and again. In 1895 Maurizio described it in *O. major* and reported cytokinesis in the zoosporangia as occurring by cell plate development, although his figure 8 shows conspicuous progressive cleavage furrows. At that time cytokinesis by cell plates had been shown in the pollen mother cells of a large number of plants, and Maurizio was doubtless influenced by these accounts in his interpretation of the process in *Olpidiopsis*. Butler likewise observed sexuality in 1907.

The most intensive cytological study of this genus, however, was made by Barrett in 1912. He illustrated and described infection of the host, its relation and reaction to the presence of the parasite, nuclear and cell division, and fertilization. The nuclei divide mitotically and simultaneously with intranuclear spindles in the developing zoosporangia. As to cell division, Barrett describes it as a simultaneous fragmentation of the protoplasm throughout the sporangia "much as described by Dangeard for the primitive cell of *Synchytrium Taraxaci*." Barrett showed the passage of the contents of multinucleate male gametes into the female thallus through a small opening in the respective walls, and the subsequent development of the zygotic resting spore. He was unable, however, to determine definitely whether the male and female nuclei fuse in pairs or all but one of each degenerate. Similarly he observed neither meiosis nor germination of the resting spores. It is thus obvious that the fundamental problems of fertilization in this genus are yet to be solved in spite of Barrett's excellent contribution.

In 1922 Schwarze studied cleavage in living zoosporangia of *O. Saprolegniae* and found it to occur by progressive cleavage. The more or less centrally placed vacuoles fuse into a large central one, and from the border

of the latter cleavage furrows arise which progress outward until they reach and cut through the plasma membrane. As the contents of the central vacuole thus escape, it disappears completely and as a result the cleavage segments contract and become visible. They may then increase considerably in size, presumably by the imbibition of water, press against each other and become polygonal. The original cleavage lines thus become less distinct as furrows, and the whole mass looks as if it had undergone simultaneous division into polyhedral bodies. Following this another contraction stage occurs, and the segments again separate and become distinct. It is doubtless the appearance of the rehydrated and subsequent contraction stages which has led most of the investigators to believe that cleavage is simultaneous throughout.

Diehl's study in 1935 on this species is apparently the latest for the genus, as far as I am aware. He studied only living material and confined his attention primarily to the effect of the parasite on the host. He found, as had all previous workers, a marked enlargement but no division of the hyphae in the region of infection, and the envelopment of the parasite in a dense layer of host protoplasm. The latter accumulates to such an extent around the chytrid thallus that the surrounding area becomes highly vacuolated and ramified by fine cytoplasmic strands which extend to the primordial utricle, as Barrett has also shown. From the appearance of his figures as well as those of previous workers there is doubtless some increase in the more viscid nitrogenous protoplasm of the host hyphae as the result of infection, but vacuolation seems to play the dominant rôle in enlargement. Neither Reinsch, Fischer, Barrett, nor Diehl shows the effect of the parasite on the host nuclei. That the host cell is stretched, however, is indicated from Diehl's plasmolytic experiments. Diehl also found that sexual reproduction occurs more abundantly at low temperatures, and that the male and female thalli may often be of the same size at the time of fusion. Furthermore, several male thalli may fuse with one female as Reinsch, Maurizio, and Barrett have also shown.

No intensive cytological study has yet been made of species of *Rozella* and *Woronina*. As noted before, Pringsheim, Cornu, and Fischer described the cytokinesis as occurring directly and simultaneously by the breaking up of the protoplasm into segments which then round up to become the zoospores. In 1890 Dangeard figured and described nuclear division in *Rozella septigina* as amitotic, but contributed nothing to our knowledge of cytokinesis. Cook and Nicholson (1933), on the other hand, describe the thallus of *W. polycystis* as becoming furrowed, and in these grooves is laid down cellulin, which thus divides the so-called plasmodium into a number of sporangia. Later the contents of these sporangia divide up into

zoospores; but Cook and Nicholson say nothing as to whether it occurs by simultaneous or progressive cleavage. Their contribution is none the less noteworthy in showing that the two cilia are attached apically rather than laterally. Most students of these genera describe the young thallus as being entirely naked or devoid of a membrane and so enveloped by the host protoplasm that the two are almost indistinguishable in the living condition. If these accounts are correct the association between host and parasite is very intimate and without any marked visible evidence of antagonism in the early stages. However, that the two protoplasts are immiscible is hardly to be doubted. None of the studies show the ultimate effect on the host nuclei and cytoplasm, although Cook and Nicholson maintain that *W. polycystis* feeds on and thus extracts the oil globules from the cytoplasm. The most conspicuous effect of these parasites is the enlargement of the host cell, but it is impossible to determine from the figures in the literature whether this is primarily due to vacuolation or an increase in the number of nuclei and the amount of dense viscid cytoplasm. The host hyphae are also stimulated to divide transversely, which suggests an attempt on their part to limit the spread of the parasite. No sexual reproduction has so far been described for these genera.

The genera *Eurychasma, Sirolpidium, Pontisma, Ectrogella, Aphanomycopsis, Pseudosphaerita, Petersenia,* and *Blastulidiopsis* must doubtless be transferred from the Olpidiaceae to this family on the basis of the discovery by Scherffel (1925), Sigot (1931), Dangeard (1933) and Sparrow (1934) that their zoospores are biciliate. The limits of these genera are not well established and defined, and some of them will perhaps with further study be merged. In fact Scherffel, Sparrow (1933) and others regard *Ectrogella, Eurychasma,* and *Aphanomycopsis* as primitive or reduced members of the family Saprolegniaceae. However, for the sake of convenience in this discussion they will be retained here and treated as valid and separate genera. Lowenthal (1905b) studied *Eurychasma Dicksonii* cytologically but figured neither nuclear nor cell division. As relating possibly to the latter he shows the mature thallus as consisting of a large central vacuole and a relatively thin peripheral scalloped layer in which the nuclei lie. This layer becomes more and more scalloped on the inner periphery, and finally the zoospores are formed simultaneously and directly. In the same year Petersen studied the development of the sporangia of the same species, and listed four stages of development which he believes are characteristic not only for this species but for the Chytridiales in general: (1) *Stade protoplasmique ordinaire* in which the protoplasm is very dense, comparatively non-vacuolate, and the nuclei are dividing; (2) *Stade globuleux* in which nuclear division has been completed and the

sporangium is filled with numerous, closely pressed, spherical globules; (3) *Stade écumeux* when the protoplasm is filled with numerous large vacuoles separated only by thin cytoplasmic bridges in which the nuclei lie, as well as in the peripheral layer; and finally, a fourth stage in which the zoospores lie peripherally disposed inside of the sporangial wall. In the transition from the third stage he noted a brief marked contraction of the protoplasm which is immediately followed by an expansion as the vacuoles disappear and the zoospore initials become visible. After the latter have been fully formed they become violently active for a short time, and then come to rest and encyst; so that the interior of the zoosporangium presents the appearance of a reticulum of polygonal segments. Sparrow (1934), on the other hand, believes that the encysted stage is the result of adverse conditions and that ordinarily the zoospores escape directly and at once. In any event the encysted zoospores germinate by escaping from their cysts in the same manner as *Achlya* and other Oomycetes. Scherffel maintains that the method of zoosporogenesis described by Lowenthal and Petersen is not characteristic of true chytrids, and hence *Eurychasma* should be removed to the Saprolegniaceae. Petersen's claim that the developmental stages which he enumerated are characteristic for the Chytridiales as a whole is certainly open to question, since his conclusions are based entirely on the study of living material. In such material, according to the author's experience, it is practically impossible to determine at what stages nuclear division is occurring. Furthermore, the peripheral disposition of the nuclei and the subsequent cleavage segments is characteristic only of such forms as have highly vacuolate zoosporangia.

The young parasite is described as naked and occupying a position adjacent to the host nucleus when young, but becoming invested with a definite membrane at maturity. The host cell becomes somewhat enlarged as Wright (1879), Rattray (1885), Wille (1899), and Magnus (1905) had previously shown, but the cytoplasm, plastids, and nucleus are not markedly distorted or injured until the parasite matures, according to Lowenthal. Pierre Dangeard and Sparrow (1934), on the other hand, describe the host cell as enlarging very shortly after infection, while the plastids soon become discolored and integrated and thus form a residual layer around the parasite.

In *Pseudosphaerita Euglenae* Dangeard (1933) describes a type of cytokinesis that is most unusual for the Chytridiales. The division of the initial nucleus is followed at once by bipartition of the thallus, and as the two resultant daughter nuclei divide the thallus undergoes division into four cells, etc. Cytokinesis thus immediately follows each division of the nuclei until the thallus or zoosporangium is divided into a large number of

polygonal zoospore segments. Dangeard shows no nuclear division figures and consequently nothing of the relation of the spindle to the formation of the cell boundaries. Hence, it is not evident from his description and figures whether cytokinesis occurs by constriction, furrowing, or cell plate formation. The time relations of mitosis and cytokinesis in this species is the same as in the meristematic tissues of higher plants, and consequently entirely different from that of all other chytrids.

In *Sirolpidium*, according to Sparrow, the elongated thallus fragments into a number of bodies which separate and become isolated zoosporangia and eventually form zoospores, but he gives no description as to how this process occurs. Similarly nothing is known of cytokinesis and nuclear division in *Pontisma* and *Petersenia*. In *Ectrogella bacillariaracearum* and other species of this genus, however, Scherffel (1925) has figured in detail the manner of origin of the zoospores. Vacuoles are more or less evenly distributed in the protoplasm at first, but as the thallus enlarges they aggregate to form a central row, as Zopf had earlier shown, and at maturity fuse into a single elongated and irregular central vacuole. From its border then develop furrows which progress outward and eventually reach the periphery. The central vacuole disappears, and shortly thereafter the cleavage segments swell to fill the entire thallus so that the lines of demarcation become less distinct and conspicuous. Scherffel is very sparing in the description of his figures and does not at any time refer to the process as progressive cleavage. It is to be noted that his figures are fundamentally similar to those of Schwarze for *Olpidiopsis Saprolegniae*, but he is apparently unaware of this paper. Scherffel stresses at length his view, as in the case of *Eurychasma Dicksonii*, that this mode of cytokinesis or zoospore formation is not characteristic of true chytrids but principally of the Saprolegniaceae, and is accordingly a criterion of relationship. He thus obviously avoids reference to the studies of Harper, Swingle, and Schwarze who have shown that progressive cleavage, whether centripetal or centrifugal, is not characteristic for a particular group but is the universal method of cytokinesis in the sporangia of the Mycetozoa, Chytridiales, Oomycetes, and Zygomycetes in general. The particular point which Scherffel is trying to stress, although he does not seem to be clearly aware of it, in my opinion, is not that cleavage is not progressive in the Woroninaceae and Saprolegniaceae but primarily centrifugal in direction because of the presence of a large central vacuole. Scherffel also figures many stages of what is undoubtedly progressive cleavage in *Aphanomycopsis*, but he does not describe them as such.

The species of *Ectrogella*, *Sirolpidium*, *Pontisma*, and *Petersenia* cause no enlargement or distortion of the host cell according to the figures of

Zopf, Scherffel, de Bruyne (1890), Petersen, and Sparrow. Zopf and de Bruyne show the host protoplasm closely aggregated around the parasites and undergoing discoloration and degeneration, but nothing further of a specific nature is known. In the early stages the parasites seem to be fairly naked, but there is no universal agreement on this point. In this group of genera sexual reproduction has been described for *E. Licmophorae* only, and according to Scherffel it is essentially similar to that of *Olpidiopsis Saprolegniae.*

Sigot's study of *Blastulidiopsis* while relating to fixed and stained material shows nothing of nuclear and cell division. The mature thallus consists of a large central vacuole, a peripheral layer of cytoplasm in which the nuclei lie, and at maturity, according to Sigot, the cytoplasm around each nucleus rounds up simultaneously to form the zoospores which then move into the central vacuole and become very active. As to host-parasite relationship, he figures *B. Chattoni* as lying in a clear space or cavity of the *Cyclops* egg and not immediately enveloped by the host protoplasm. This clear space may possibly be a digestion cavity, as in the case of plants parasitized by members of the Erysiphaceae, into which ferments flow and digest the host protoplasm. On the other hand, it may be nothing more than the result of shrinkage from fixation.

The genus *Pseudolpidium* as recognized at the present time has been studied only in the living condition, and as a consequence little or nothing specific is known about nuclear and cell division. Sexual reproduction has never been observed. Butler followed the maturation of the zoosporangia in several species but does not describe cytokinesis, while Cornu and Fischer, as noted before, figure vacuolated zoosporangia and report the formation of zoospores as occurring directly. The effect of the parasite on the host hyphae is quite marked. They enlarge and become more or less vesicular as when parasitized with *Olpidiopsis*, *Rozella*, and *Woronina*, and may be stimulated to divide transversely. According to the descriptions of Cornu, Fischer, and Butler, the relation between the young parasite and the host protoplasm is apparently very similar to that of these genera and equally as little known or understood.

In the genus *Pythiella*, parasitic on *Pythium*, Couch (1935) has given an excellent illustrated account of cytokinesis and sexual reproduction from a study of living material, but he contributes nothing about nuclear division and the behavior of the gamete nuclei during fusion. Cell division in the zoosporangia is progressive and centrifugal from the border of a large central vacuole, as Schwarze has described it in *Olpidiopsis Saprolegniae.* The zoospores are somewhat reniform in shape with two laterally attached cilia. After escaping from the zoosporangium they lie quiescent

at the mouth of the exit tube for some time and then germinate in the same fashion as in *Achlya*. Sexual reproduction consists of the fusion of a small male and a large female thallus through a short conjugation tube or canal. More than one male thallus or gamete (fig. 25) may fuse with the female thallus as in *O. Saprolegniae*. After fertilization the zygote rounds up and becomes invested with a comparatively thick wall. As to its effect on the host, *Pythiella* causes conspicuous hypertrophy of the *Pythium* hyphae in the region of infection, but no transverse septation, according to Couch.

This résumé shows that nuclear division has been well figured and described in only one genus; cytokinesis in five, and sexual reproduction in three genera. The majority of species cause marked enlargement of the host cell, but the cytological changes involved and the details of hypertrophy as well as the intimate relation of the parasites and host protoplasm are but imperfectly known.

OLPIDIACEAE

The members of this family have not been studied as intensively as those of the Synchytriaceae, although the number of genera is considerably greater. None the less, the same degree of uncertainty and conflict of opinion as to methods of nuclear and cell division occurs in the literature, and the accounts of sexual reproduction are equally diverse. In 1878 Woronin postulated on the basis of Cornu's (1872) and Nowakowski's (1877b) demonstrations of sexuality in *Olpidiopsis* and *Polyphagus* that the resting spores of *Olpidium Brassicae* arise from the fusion of motile isogametes, and six years later Fisch (1884) reported it to occur in *Reessia amoeboides*, which is perhaps a species of *Olpidium*. Fisch also described fusion of unequal thalli to form the resting spores in what has since been recognized as *Pseudolpidiopsis parasitica*. In the same year Zopf described and figured fusion of small male and large female thalli to form resting sporangia in *Pseudolpidiopsis Schenkiana*.

This type of sexual reproduction was confirmed by Fischer in 1892 and Wildeman in 1895 for other species of the same genus, and since that time has been observed again and again (Butler, 1907; Tokunagu, 1933). Very little was contributed to the cytology of the strictly olpidiaceous species of this family, however, until 1910, when Griggs described a new species, *Monochytrium stevensianum* in which the young uninucleate thalli fuse within the host cell to form binucleate, thick-walled resting spores. Although this is doubtless a species of *Olpidium* the present binomial will be retained in this discussion. Fusion of the gametic nuclei is apparently delayed until shortly before germination, although it was not observed by Griggs. The thalli which do not fuse develop into thin-walled evanes-

cent zoosporangia. During the early growth of such thalli the primary nucleus fragments by constriction into four daughter nuclei, but no further multiplication occurs until the incipient zoosporangia have attained almost mature size. Then, according to Griggs, follows a period of rapid amitosis, although he is not certain that this is the universal method of nuclear division. Nothing is said or known as to the mode of cell division. The parasite causes only slight hypertrophy of host cell, does not injure it perceptibly for a long time, and lives to some extent in a mutualistic relationship with its host, according to Griggs. In the following year Němec (1911) reported a new species, *Olpidium Salicorniae*, with both evanescent zoosporangia and smooth thick-walled resting spores. In the former the nuclei were found to divide mitotically and simultaneously. The resting spores were either uni- or binucleate, and in the former cases the nuclei always appeared peculiarly constricted, like two nuclei in the process of fusion. From such appearances Němec postulated the possibility that the resting spores are formed by the fusion of two small naked uninucleate thalli in the host cell, as in *M. stevensianum*, and that the binucleate character and constricted nuclei of the resting spore might be stages in nuclear fusion. During the next year Němec (1912) also reported and figured regular mitosis of the nuclei and progressive cleavage in the evanescent zoosporangia of *O. Brassicae*. The cleavage grooves or furrows usually begin at the periphery and progress inwards, but if the zoosporangium has a large central vacuole centrifugal furrows may also form.

At the same time Kusano (1912) published an extensive account on the cytology of a new species, *O. Viciae*, in which he reports the fusion of motile isogametes to form binucleate motile zygotes which infect the host cell and develop into smooth, thick-walled resting spores. Karyogamy or nuclear fusion, however, does not occur until the spores begin to germinate. In the growth and maturation of the resting spore the gamete nuclei undergo remarkable changes, according to Kusano. The nucleole migrates to the outside of the nuclear membrane, and the nucleus then buds at the tip of the nucleole. This bud gradually increases in size and attains almost the same form, volume, and appearance of the main body, forming thus a dumbell-shaped structure with the nucleole between. The membrane of the bud then disintegrates, and the contents pass into the central vacuole of the spore. Kusano regards this budding as an unusual type of amitosis, since in the process the median nucleole is divided into two halves, one going into the bud and the other returning to the mother nucleus. However, the subsequent degeneration of the bud indicates that this is only a peculiar type of nuclear extrusion. The first stage of germination of the resting spore is initiated by the conjugation of the gamete nuclei. The

diploid fusion nucleus then divides, but Kusano was unable to determine conclusively whether or not the first division is indirect and meiotic. He figures two metaphase spindles of the daughter nuclei, but failed to find any others and is therefore uncertain as to the universal mode of division. The zoospores which fail to fuse infect the host and form zoosporangia. During this development the nuclei multiply by an unusual type of amitosis in which the nuclear membrane breaks down and the chromatin granules as such disappear. New nuclei are then formed, which subsequently divide mitotically, as is well shown in his figures. In *O. Viciae*, thus, mitosis follows amitosis in the development of the zoosporangium. Kusano is quite ambiguous as to the method of cell division, but states definitely that it is not progressive cleavage. "Clear spaces appear in the cytoplasm all at once between each two nuclei, cutting off the cytoplasm in as many polygonal parts as the nuclei." In 1922 Němec described fusion of motile isogametes in *O. Brassicae*, confirming thus Woronin's assumption that the resting spores in this species are formed sexually. In the same year Sawada (1922) likewise reported the same type of sexual reproduction in *O. Bothriospermi*. In 1927 Guyot published a lengthy account on the systematic position, host range structure and development of *Asterocystis radicis* but contributed nothing of significance cytologically.

Schwartz and Cook (1928) reported a new species, *O. radicale*, in which the zoospores become motile again after entering the host cell. Later on fusion between small and large thalli, which are designated as antheridia and oogonia, occurs, which is followed by immediate karyogamy and the development of a thick wall around the zygote or resting sporangium. Although they did not actually observe the processes, they none the less describe the fusion nucleus as first dividing meiotically and later homeotypically a great number of times to form a large multinucleate zoosporangium. The thalli which fail to fuse develop into thin-walled evanescent zoosporangia. Cell division or zoospore delimitation in both types of sporangia occurs by vacuolation of the cytoplasm, according to their description. It is to be particularly noted that their observations were not made from fixed and sectioned material but only from mounts stained in toto. They failed to observe conclusive cases of cell fusion, karyogamy, and meiosis, and although they figure a few mitoses they are not certain but that amitosis may also occur. Their whole account of the life history and cytology of this species is woefully uncertain as to the critical stages, and seems to be largely a description by analogy to bring it in line with those of other observers.

In 1929 Kusano described the life history of *O. Trifolii* in which he found copulation of motile isogametes to form resting sporangia as in

O. Viciae. Fusion occurs only between gametes from different zoosporangia. In this species Kusano contributes nothing to our knowledge of nuclear and cell division. In 1931 de Wildeman described a new species, *O. Stigeoclonii* which causes marked hypertrophy of the infected *Stigeoclonium* cells. They may become five and seven times their normal diameter and length respectively and are more or less completely filled by the chytrid zoosporangia. The presence of the parasite, however, does not stimulate them to division as has been noted above in cases of infection by *Synchytrium*. The young parasite takes up a central position in the cell adjacent to and partly surrounded by the band-shaped chloroplast, and as it enlarges the latter is compressed and flattened against the cell wall. The plastid is not particularly injured at first and does not become disorganized until after the parasite has matured, according to de Wildeman. Cytokinesis or the formation of the zoospores is quite unique and so unlike that of other species that one is inclined to regard it as abnormal. The protoplasm of the zoosporangium oozes out of the neck, and successively from this mass individual rounded globules separate or are pinched off one by one. Cilia may develop while the globules are still attached to central mass, but they are lost almost at once so that the zoospores never swim about actively. Movement is accordingly only amoeboid.

Miss Sampson recorded a new species, *O. Agrostidis*, in 1932, and followed carefully its developmental stages in the host cell. No fusion of gametes was observed, but since the resting spores are invariably binucleate until maturity she believes they originate by copulation of zoospores. Nothing is reported on the type of nuclear and cell division, although Miss Sampson gives a number of figures of fixed and stained thalli. The latest contribution to the cytology of the *Olpidium*, as far as I am aware, is by Cook and Collins in 1935 on *O. majus*. They reported fusion of thalli in the root hairs of cucumber to form diploid resting spores as in *O. radicale*. They did not, however, actually observe the process of fusion, but only figured resting spores with a smaller empty thallus attached, which is interpreted as the male gamete. No nuclear fusion was seen, but karyogamy apparently occurs at once, since no binucleate spores were found. Neither was nuclear division observed. Cook and Collins described cleavage furrows which delimit the zoospores, but it is not obvious from either their drawings or account whether the furrows occur simultaneously throughout or develop progressively.

In another genus, *Cystochytrium*, whose taxonomic position and relationship with the chytrids is still obscure but which nevertheless resembles *Olpidium* to some degree, Cook (1929, 1932) described and illustrated clearly defined simultaneous mitotic figures in the equatorial or meta-

phase stage. No other stages were observed, and nothing further is known regarding cytokinesis, host-parasite relationship, and sexual reproduction.

In *Pleolpidium*, *Septolpidium*, *Pleotrachelus*, *Plasmophagus*, *Sphaerita*, and *Nucleophaga* nothing is known of nuclear division, and the fragmentary accounts of cytokinesis are based primarily on living material. The young protoplast of *Pleolpidium*, according to Cornu, Fischer, Butler and others lies more or less naked in the host hyphae and completely surrounded by but immiscible with their protoplasm. As it grows in size the host hyphae and sporangia enlarge and eventually become vesicular in the region of infection, but with the exception of parasitism by *P. irregulare* (Butler, 1907) the former are not stimulated to divide, according to the descriptions in the literature. At maturity the parasite completely fills the host vesicle or gall, and its wall or membrane is reported to fuse with or become so closely appressed to the host wall that the two are indistinguishable. None of the species have been studied cytologically from fixed and stained material, so that the exact relation of the host and parasite walls as well as the nuclei and cytoplasm is yet unknown. No sexuality has yet been described in this genus, but according to Butler a number of protoplasts from separate and individual zoospore infections may fuse inside of the host and form a plasmodium. He does not, however, state whether or not any nuclear union results from such fusion. In *Septolpidium* the division of the elongated thallus into a linear series of zoosporangia is progressive and centripetal, according to Sparrow (1936b), while the zoospores are delimited by progressive cleavage.

It is doubtless questionable whether all species of the genus *Pleotrachelus* should be included here in light of Petersen's (1905) and Sparrow's (1936b) observations that the zoospores of *P. Andreei* are biciliate. Since this character has been found in only one species, *P. Andreei* should perhaps be removed from the genus as Sparrow has recently done. *Pleotrachelus fulgens* on *Pilobolus* has much the same effect as *Pleopidium*, *Olpidiopsis*, and *Pseudolpidium* on the host cells. The infected host hyphae and sporangia enlarge and become somewhat vesicular and may occasionally be delimited by cross septa, according to Zopf's figures. It is not obvious, however, whether the enlargement is accompanied by an increment of host protoplasm or by marked vacuolation. At any rate the host protoplasm becomes conspicuously discolored and may change to yellow and intensely orange or golden red with the growth and development of the parasite. In the other species of *Pleotrachelus* described by de Wildeman (1893), Petersen (1905), Lund (1930) and Sparrow (1934), hypertrophy of the host cell may be very slight or lacking entirely, according to the reports. In a species closely similar to *P. Andreei* Sparrow found small com-

panion cells or thalli attached to the developing and mature resting spores, and although he did not see actual fusion he believes the latter are formed as the result of fertilization.

The genus *Plasmophagus* has been even less studied than the two preceding ones and contains only a single species, *P. Oedogoniorum*, which was first reported by de Wildeman in 1895. He described it as almost completely filling the *Oedogonium* cells at maturity and being to a certain degree indistinguishable from the host protoplasm. Its walls, however, always remain distinct from those of the host. According to de Wildeman the nuclei of the host cell may continue to divide for some time, but cell division is inhibited. As a result greatly elongated and multinucleate cells are formed, and in *Tribonema* these may enlarge to almost twice their normal diameter, according to Sparrow (1933). The host protoplasm is almost completely consumed by the parasite at maturity and may occasionally form a thin discolored disintegrating residual layer around the periphery. Neither de Wildeman nor Sparrow contributes anything to our knowledge of nuclear and cell division of the parasite.

The genus *Sphaerita* has been recorded rather extensively, particularly by protozoologists, since its discovery by Dangeard in 1886, but in spite of this we know very little as to the cytology of its critical stages. While a large number of these studies (Chatton and Brodsky, 1909; Dobell, 1919; Dobell and O'Connor, 1921; Cunha and Muniz, 1923; Kessel, 1924a and b; Brumpt, 1926; Becker, 1926; Li-Yuan-Po, 1928; Jirovec, 1933; Crouch, 1933; Brumpt and Lavier, 1935) relate to fixed and stained preparations they nevertheless fail to show anything significant about the processes of nuclear and cell division. These studies have been primarily concerned with the cytology of the host, and their discourses on *Sphaerita* and *Nucleophaga* relate only indirectly to isolated stages of development which occurred sporadically in their material. Dangeard (1886, 1889, 1895) described cytokinesis in *S. endogena* as a simultaneous breaking up or fragmentation of the protoplasm in the zoosporangia into polyhedral segments, and in 1889 showed stages in the fusion of uniciliate zoospores to form biciliate zygotes (fig. 8, pl. 3). He was not certain, however, that resting spores always result from such fusion, and finally in 1933 after describing the biciliate character of the zoospores of *Pseudosphaerita* he raised the question whether the zygote previously described for *Sphaerita* may be nothing but the zoospores of the other genus. In the same species apparently, Nägler (1911) describes the nucleus as undergoing constriction and promitosis, and later fragmentation, while his account of cytokinesis and zoospore formation is very confusing and difficult to interpret in light of Dangeard's description. Chatton and Brodsky failed to observe zoo-

spore fusion in a species of *Sphaerita* which they found in *Amoeba limax*, but nevertheless believe it occurs in this form also and is general for the whole genus. As to cell division, they describe the cytoplasm as condensing around the nuclei and secreting a thin spore membrane. Penard (1912), on the other hand, while studying the same species apparently saw what he claims to be conjugation of quiescent (fig. 33) swarmspores. In *S. endamoeba* Becker describes and figures the nuclei as deeply staining bodies lacking membranes or any other structural differentiation, which divide more or less simultaneously in bipartite fashion without any evidence of spindle figures. When the sporangia are mature the nuclei enlarge somewhat, stain less intensely, develop membranes, and become the zoospores directly. This account of Becker is quite unlike that of any previous and subsequent investigator, and it is not improbable that his unusual description is based on poorly fixed and stained material. Crouch on the other hand, describes and figures the thallus of *S. trichomonadis* as undergoing more or less regular and simultaneous bipartition to form the spores, while the nucleus is represented as a small black dot surrounded by a clear space.

The relation between *Sphaerita* and its host is rather unusual in many respects, since the parasite does not kill the active host cell until quite late. *Sphaerita endogena* in *Euglena* may often reach maturity, according to Dangeard (1895) and produce zoospores before any marked effect is visible in the host cell, and in many cases the *Euglena* may return to normal appearance and activity after the parasite has been expelled. Puymaly (1927), however, denies this and maintains that the ciliary motion of *Euglena* almost always ceases after infection, but spasmodic action may continue until the zoospores of the parasite are liberated. He furthermore describes the zoospores as biciliate with a long posterior and a shorter anterior cilium, and fails to confirm Dangeard's account of fusion. In light of the fact that *Pseudosphaerita* parasitizes the same host and has biciliate zoospores, according to Dangeard (1933), it is not improbable that Puymaly may have confused this organism with *Sphaerita*. Sometimes the hosts encyst and thus inhibit the development of the sporangia. Usually, however, the host nucleus becomes spongy, the chromatin condenses, the nucleole disappears, the chromatophores turn yellow and disintegrate, while the cytoplasm becomes thinner and less in quantity, and finally the cell bursts. In *Nuclearia* and *Heterophyra* (Dangeard, 1886a and b), on the other hand, *S. endogena* has but little deleterious effect at first, and the host cells may continue to divide so that the parasite is distributed to one of the daughter cells. Later, however, it inhibits division. In the cells of various amoebae, according to Chatton and Brodsky, Mattes

(1924), Becker and Dobell, *Sphaerita* has no marked toxic effect, and injures primarily by encumbering and impoverishing the host. In its young stages the ingested spore is indistinguishable from ordinary food particles lying in the vacuoles, and the membrane around the developing parasite is scarcely perceptible. The amoeboid motion of the host continues unchanged for a considerable length of time, but gradually becomes slower. At the same time the amoeba becomes more spherical in shape with the pseudopods assuming a radial direction. The contractile vacuole continues to function for a long time, but eventually its rhythmic pulsation ceases and it disappears altogether. However, the fundamental structural appearance of the cytoplasm and nucleus is retained for a long time, and the nucleus may often remain intact after the rest of the host has been destroyed. According to Penard the amoebae may often rid themselves of the parasites like extraneous food particles and continue to live normally.

Nucleophaga, a questionable genus which may perhaps be related to *Sphaerita*, was first described by Dangeard in 1895 as a parasite in the nuclei and nucleolus of various rhizopods, and since that time a large number of additional species have been reported by Gruber (1904), Penard (1905), Mercier (1907, 1910), Elpatiewsky (1907), Doflein (1907), Epstein (1922), Brug (1926), Kirby (1927), Lavier (1935a, 1935b), Brumpt and Lavier (1935) and others. According to Dangeard and subsequent workers the parasite enters the nucleus and nucleole and appears first as a small vacuole with a central granule which represents its nucleus. It grows at the expense of the chromatin, karyolymph, and nucleole and soon fills the nuclear cavity, and with subsequent development the nucleus becomes greatly distended and enlarged. In cases where the nucleole is not infected it is shoved aside by the developing parasite and flattened against the nuclear membrane into a crescentic disk. Later it may break up into fragments and disintegrate or be partially consumed. At maturity the entire thallus of the parasite is transformed into zoospores. The manner in which this occurs is as yet little understood in spite of all the studies which have been made. Dangeard describes cytokinesis as taking place simultaneously, but none of the subsequent investigators have contributed anything further on the process. Likewise nothing is known as to the method of nuclear division. Doflein was the first to observe the motility of the zoospores, and described their fusion to form biciliate zygotes. These encyst and later on germinate to form amoeboid thalli. Doflein's account is the only report so far of sexual reproduction in this genus.

As to the effect of *Nucleophaga* on the activity of its host, most investigators are agreed that parasitized amoebae show little if any immediate effect. Dangeard and Gruby in particular report that the host moves about

and feeds normally even when the nucleus is enormously distended and filled with parasites. Normal activity may continue for some time after the nucleus has been completely destroyed, but the pseudopodial movement gradually becomes slower and ceases entirely. The amoebae eventually round up, and as the zoosporangia of the parasite open and the zoospores escape the host cell begins to disintegrate. *Nucleophaga* feeds primarily on the chromatin reticulum and nucleole, but according to Dangeard it may also draw nourishment from the cytoplasm outside. In this process the nuclear reticulum is gradually absorbed and disappears as such, while the nucleole may become less basophilic and finally disintegrate into fragments.

Whether *Blastulidium*, *Endoblastidium*, *Coelomycidium*, *Chytridiopsis*, and *Chytridioides* belong in this family or are to be considered as chytrids at all is highly problematical. In the last three decades many protozoologists have tentatively assigned a number of genera and species to this family which do not appear to fit into any groups of the Protozoa. In some respects their development and life histories are similar to *Olpidium*, *Nucleophaga*, and *Sphaerita*, but they are as yet imperfectly known. *Blastulidium paedophthorum* was first described as a parasite of eggs and young embryos of *Daphnia obtusa* by Perez in 1903 and 1905. Its thallus is oval with a thin limiting membrane and a large central vacuole, and at maturity is divided up completely, as in the Olpidiaceae, into polyhedral segments which round up to become spores. Perez does not report any evidence of sexuality nor describe nuclear and cell division, but his figures suggest at least that the latter process occurs by progressive cleavage. He placed this species in the family Haplosporidiidae, but in 1905 Caullery and Mesnil transferred it to the Coelosporidiidae. Finally in 1908 Chatton found the uniciliate zoospores, and on this basis primarily assigned the genus provisionally to the chytrids in the neighborhood of the Olpidiaceae.

Coelomycidium attacks and destroys the proximal adipose tissue of the larvae of *Simulium*, according to Debaisieux (1920), and is strikingly similar to *Olpidium* in vegetative structure and asexual reproduction. Debaisieux was unable to differentiate the successive stages of mitosis in his fixed and stained material of the incipient zoosporangia, but nevertheless describes division as indirect and bipolar and figures numerous nuclei which are undoubtedly in various phases of division. He describes the thallus as a plasmodium and figures a number of stages of progressive cleavage in the formation of the zoospores. No sexual reproduction was observed, and the thick-walled hyaline resting spores which appear in the winter may occur singly or in groups held together by a common membrane. They may be uni- or multinucleate, and the nuclei are described

as dividing by bipartition, although Debaisieux found one case of indirect division. From his figures it seems probable that karyokinesis is indirect throughout and that the stages of bipartition are abnormal, as Rytz observed for *Synchytrium*.

Endoblastidium was first described by Codreanu in 1931 as a parasite in the coelomic cavities of larvae of *Baetis Rhodani* and *Rhitogena semicolorata* and bears considerable resemblance to *Coelomycidium* and *Olpidium* in some respects. It is primarily an inhibitory parasite, according to Codreanu, which suppresses the growth and development of the organs, and at maturity is ejected from the anus of the host. In its vegetative stage the thallus is somewhat irregular in shape and plasmodium-like, but becomes oval and surrounded by a fairly thick membrane at maturity. After ejection from the host it is completely divided up into uniciliate oval zoospores which escape through an elongated slit or cleft. Codreanu figures a number of nuclear changes which suggest early prophases of mitosis, but does not describe the manner of cytokinesis or report any evidence of sexuality.

Chytridiopsis, according to Schneider (1884) and Leger and Duboscq (1902, 1909), is a parasite of the intestinal cells of *Blaps mucronata* which causes marked distention of the epithelial cells and leads to the destruction of their cytoplasm and nucleus. The thallus begins as a small uninucleate oval corpuscle in the basal part of the cell which increases rapidly in size and at maturity divides into a large number of spores. In this process the nucleus of the host cell becomes flattened on one side and begins to degenerate, and when zoosporogenesis is complete the degenerated host cell and parasite are expelled from the epithelial layer. The spores are thus liberated into the intestine, become amoeboid, and infect other cells. No flagellated stage has so far been observed. According to Leger and Duboscq certain thalli form amoeboid micro- and macrogametes which fuse to form a zygote. The latter develops into thick-walled resting cysts which are eventually expelled with other excrement from the intestines. Neither of the observers describes nuclear and cell division, and their account of sexuality is rather obscure and unconvincing. Schneider considered this genus as a primitive chytrid, while Caullery and Mesnil, regarded it as a member of the family Haplosporidiidae. Leger and Duboscq, on the other hand, believe it to be related to primitive mycetozoa resembling the Monadinae. *Chytriodes Schizophylli* is closely similar to *Chytridiopsis socius* according to Tregouboff (1913) and parasitizes the epithelial cells of the middle intestine of *Schizophyllum mediterraneum*. The young thallus is more or less spherical with a single nucleus which is represented by a granule and completely devoid of a membrane. As it increases in size the nuclei mul-

tiply, and at maturity it is cut up into uninucleate polyhedral segments, which become amoeboid and are expelled from the sporangium and the infected epithelial cell. No cilia have so far been observed and Tregouboff shows nothing of nuclear and cell division. As to its relation to the host cell, *Ch. Schizophylli* distends the latter considerably and depresses the nucleus to one side. However, no marked changes occur in the nucleus and cytoplasm according to Tregouboff's figures and description. A thick-walled resting spore or cyst is frequently formed, and Tregouboff believes it arises from the union of small and large fusiform gametes, although he did not observe the process of fusion. The young zygote or cyst increases in size, but instead of giving rise to spores directly divides into locules or compartments in a fashion very suggestive of sporange formation in the sorus of *Synchytrium*. Each compartment gives rise to spores, but in the process the partitioning walls are gradually resorbed, so that the mature resting cyst is unilocular and filled with round spores. It is quite obvious from these descriptions of Schneider, Leger and Duboscq and Tregouboff that *Chytridiopsis* and *Chytridioides* are very questionable chytrid genera, and in our present state of knowledge they should perhaps be excluded entirely.

From this summary of the literature on the Olpidiaceae it is apparent that no adequate and detailed description of nuclear and cell division has yet been given in any of the numerous species. The nuclei have been described as fragmenting, budding, and dividing both directly and indirectly, and for the latter type only a few isolated equatorial plate stages have been shown. For cytokinesis we find, with only one or two exceptions, scarcely more than the general descriptive terms. "Clear spaces appear in the cytoplasm . . . cutting the cytoplasm into as many polygonal parts as the nuclei"; "whole structure becomes cut up into a large number of uninucleate zoospores"; "contents divide up into a large number of separate areas," etc., without any convincing figures to accompany them.

In no species has meiosis yet been described, although fertilization has been reported in more than half a dozen members of this family. As to the types of sexual reproduction, fusion between motile isogametes has been reported in five species, and copulation of small male and larger female thalli or so-called antheridia and oogonia has been described for four additional members, excluding *Chytridiopsis* and *Chytridioides*. In all of these cases the gametes are uninucleate at the time of fusion. Sexual reproduction in *Pseudolpidiopsis Schenkiana* and other species of this genus, however, is identical with that of *Olpidiopsis Saprolegniae* of the family Woroninaceae in which the gametes are multinucleate, but no cytological study has yet been made to determine whether or not it is similar in this latter

respect also and that the gametic nuclei fuse in pairs. Karyogamy may immediately follow plasmogamy, but in the majority of described species it is delayed until germination of the zygote or resting spore.

The host-parasite relationship in this family has not been studied as intensively as in the Synchytriaceae, with the exception perhaps of *Sphaerita* and *Nucleophaga*, but it is apparent from the literature that the reaction of the host in the majority of cases is not as marked as in the latter family. In most species which have been studied cytologically the host cells and cell organs have been reported to enlarge to several times their natural size, particularly if young at the time of infection, and the adjacent healthy cells become greatly compressed, distorted, and elongated. The mass enlargement of an infected region is thus due primarily to hypertrophy of the parasitized cells. However, the production of galls is not nearly as pronounced as in the Synchytriaceae. Furthermore, the host nuclei are not conspicuously enlarged, distorted, nor do they become reticulated and furrowed. No nucleolar growth and fragmentation has been described as the result of parasitism. In *Monochytrium stevensianum*, according to Griggs, the host nucleus retains its finer structure and staining reaction until the cell is killed, and the relation between host and parasite appears to be somewhat mutualistic. In *Olpidium Viciae* and *O. Trifolii* Kusano reports the host nucleus as becoming only slightly enlarged or compressed, while the increased cytoplasm is scarcely perceptible. In *O. Sphacellarum*, according to Sparrow (1936) the young thallus attaches itself to the nucleus of the host cell, and in the lateral branches of *Sphacelaria* causes pronounced swelling of the infected region. Similar swellings or hypertrophy of the infected host cells has been reported by de Wildeman (1895) for *Pseudolpidiopsis Zopfii* and *P. fibrillosa*. It is not certain, however, that these species belong in the genus *Pseudolpidiopsis*, since the zoospores and the number of cilia which they bear have never been observed.

In *Olpidium Salicorniae* Němec has described and figured marked hypertrophy of the infected host cells also. Furthermore, as the germ tube penetrates the host wall, the latter develops internal cellulose excresences or caps which envelop it and may become elongated into tubular canals which traverse the lumen of the cell. These excrescences are quite similar to those described by Scherffel for algal cells parasitized by rhizidiaceous species, and their formation is apparently a reaction on the part of the host which prevents the entry and spread of the parasites. Cellulose caps of a similar nature but reduced in size have also been described by Griggs for *M. stevensianum*.

RHIZIDIACEAE

This family contains without doubt the largest number of genera and species, and is the second of the chytrid families in which indisputable sexuality was first observed. In 1877 (b) and 1878 Nowakowski described in detail the life history and development of *Polyphagus Euglenae* and *P. parasiticus* and showed that the spiny and smooth-walled resting sporangia are formed by the conjugation of male and female thalli of unequal size. The smaller or male thallus sends out a long conjugation tube which comes in contact with the larger female thallus, and a zygospore is formed at the tip of this fusion canal. Very little further was contributed to the cytology of this family until 1898 and 1899, when Wager gave a short preliminary account of the structure and behavior of the nuclei in resting spore formation in *P. Euglenae*. The male nucleus and cytoplasm enter the incipient zygospore or swelling first, and are then followed by the female protoplast. The former is at first smaller and less chromatic, but with continued growth of the zygospore it gradually attains the same size and appearance as the female nucleus. Karyogamy is delayed until germination. The zygospore forms a tubular vesicle or sporangium into which both gamete nuclei migrate and finally fuse. In the next year Dangeard (1900) published an extended cytological account of the same species in which he showed that the nuclei of the zoosporangia divide mitotically and simultaneously but without centrosomes or astral radiations. The division spindle is completely intranuclear, and after karyokinesis is completed the sporangium is cut up into polyhedral segments simultaneously. He confirmed in general Wager's description as to nuclear behavior during fertilization, but maintained that the female nucleus was the first to enter the incipient zygospore.

Finally in 1913 Wager gave his completed report on the cytology of *P. Euglenae*. Nuclear division in both zoosporangia is mitotic and simultaneous with intra-nuclear spindles. Wager describes the structure of the resting nucleus and the later stages of mitosis but contributes very little to our knowledge of the prophases. No evidence of centrosomes was observed in the prophases, but after the spindles have been fully formed, they push through the nuclear membrane at the poles, and polar radiations then appear in the cytoplasm. His account of cell division in the sporangia is not particularly elucidating as to the exact manner by which it occurs, but from his figures together with the description given it appears to be identical to the process of progressive cleavage as described by Harper for *Synchytrium decipiens*. The two gametic nuclei in the young zygote begin to extrude two large separate masses of chromidia which ultimately

fuse in the cytoplasm. In this process the nuclei become quite small, and Wager describes them accordingly as generative nuclei. As the zoosporangium is formed in germination they pass out of the spore and fuse likewise. Thus, according to Wager, double fusion occurs in *P. Euglenae*—chromidial fusion in the spore and nuclear fusion in the sporangium. The nucleus is thus regarded as having a dual character: the chromidia represent the trophic or somatic element and the small nuclei the hereditary or generative element. Wager thus adopts Hertwig's, Schaudinn's and Goldschmidt's "chromidial hypothesis" to explain the peculiar behavior of the nuclei in the germination of the zygospore of *Polyphagus Euglenae*. In 1925 Scherffel confirmed the observations of Nowakowski (1878) on *P. parasiticus* as to its development and sexuality, but contributed no further cytological details.

According to Nowakowski, Fisch (1884), Dangeard (1900) and Scherffel the prosporangium may encyst and develop into a resting spore without fusion. Such spores, according to Dangeard, may be readily distinguished from the zygote by their uninucleate character, thin, single-layered wall, and fewer, minute refractive globules. He and Scherffel regard this as parthogenetic development. The latter has also figured smaller spiny spores which appear as if they were the encysted prosporangia of the small so-called male thalli. If such thalli are definitely male it is obvious that the small spores have been formed androgenetically.

Fisch has cast considerable doubt on the sexuality of the resting spores by the observations that in rich cultures of *Polyphagus* numerous thalli may anastomose and form an intricate network with numerous spores between them. He believes that fusion between two thalli is either fortuitous and accidental or a means of nourishing and rejuvenating a small weak thallus which is incapable of independent existence. Zopf (1890, p. 561) takes the same viewpoint. This conception, however, was formed before anything was known concerning the behavior of the nuclei in fusing thalli.

In the meantime Lowenthal (1905) had described a new Chytridium-like, operculate species, *Zygorhizidium Willei*, whose sexual reproduction is somewhat similar to that of *P. Euglenae*. Small uninucleate male and larger female thalli or gametangia occur on the same host cell; and the former then develops a lateral, continuous conjugation tube of varying length in the direction of the female, with which it eventually fuses. The uninucleate protoplast of the so-called antheridium migrates through the tube into the larger thallus, and plasmogamy occurs at once. Nuclear fusion apparently does not immediately follow, since Lowenthal has observed binucleate zygotes long after fusion had occurred. In any event a

thick wall soon envelops the diploid cell, and the smooth mature resting spore is formed. Both Lowenthal and Scherffel (1925) believe that the female thallus may occasionally encyst and develop into a resting spore parthenogenetically, and it is not improbable that the male thalli may likewise do so. The sexuality of these so-called male and female thalli does not appear too strongly fixed, since if they fail to copulate they may develop directly into vegetative zoosporangia. As to where in the life cycle sex segregation occurs nothing is known. Neither Lowenthal nor Scherffel has determined whether the zoospores which develop into male and female thalli respectively come from the same or different zoosporangia.

Scherffel maintains that *Zygorhizidium* is but a form of *Chytridium* which develops its resting spores sexually and extramatrically. Accordingly he proposes the alternative sub-generic name *Ectochytridium* for it and includes tentatively *Chytridium Confervae* Wille, *Ch. chaetophilum* Scherff., *Ch. Characii* Scherff. and *Ch. Spirotaeniae* Scherff, with it.

Lowenthal describes nuclear division in the zoosporangia as being mitotic, although he did not observe any sharp and well-defined figures. As to cell division, the cytoplasm first clumps or concentrates around the nuclei, and then the whole protoplast breaks up into uninucleate segments.

In 1907 Serbinow described the life history, development, and sexuality of a new completely extramatrical species, *Sporophlyctis rostrata*, which may well be considered in this place, although its taxonomic position and relationship are obscure. Sexual reproduction occurs by the fusion of a small male and a larger female thallus. The smaller attaches to the larger, and the content of the latter migrates through a pore into the former, which then forms a spiny outer wall. Karyogamy follows plasmogamy at once. The thalli which do not fuse develop into evanescent sporangia and form uniciliate but non-motile spores. These are liberated when the sporangium wall breaks and develop into thalli. Serbinov does not figure or describe nuclear division, meiosis or cytokinesis. Petersen established a new rhizidiaceous genus, *Siphonaria*, in 1910 for which he described and figured an unusual type of reproduction. "A rhizoid-like thread from a sporangium fuses with a similar thread from another," forming presumably a conjugation canal through which the content of one sporangium passes completely into the other. The latter then becomes larger and invests itself with a thick, brown, smooth wall. Sparrow confirmed this account of sexual reproduction in *Siphonaria variabilis* in 1935 and extended it to the closely related genus *Rhizoclimatium* also, and in 1936 Ledingham described sexual fusion through the rhizoids of separate thalli in *Rhizophidium graminis*. However, he only observed the intermingling and contact of rhizoids and did not actually see fusion of the two protoplasts.

Ledingham also found fusion of zoospores in this species but he apparently does not believe that this leads to the formation of resting spores. Recently Sparrow (1936c) has presented data to show that the resting sporangia of *Diplophlyctis intestina* are formed as the result of fusion of two thalli by anastomosis of their rhizoids and the migration of the protoplasm of one into the other in the same manner as in *Siphonaria* and *Rhizodosmatium*. Although I have had this species under more or less continual observation for a number of years I am unable at the present time to confirm his report. Minute empty thalli have frequently been found in the vicinity of young developing resting sporangia, but it has been impossible to determine with certainty anastomosis between the rhizoids because of the opacity of the host cell wall. More favorable methods of growing this chytrid must be developed before Sparrow's contention can be conclusively settled. As has been described in a previous paper (1936a) I have frequently found individual thalli bearing micro- and macrozoospores respectively, and there is thus the possibility that *D. intestina* is heterothallic. So far, however, no fusion between the two types of motile cells has been observed, and it is likewise probable that the two thalli represent distinct species.

With the exception of these studies on *Polyphagus*, *Zygorhizidium*, and *Sporophlyctis* very little additional has been contributed to the cytology of this widely diverse group. Němec described and figured a few curious uninucleate, rhizidiaceous and amoeboid thalli of his new species, *Entophlyctis Salicorniae* in 1912, about which there is still considerable doubt. Subsequent papers have dealt primarily with morphological development and occasional records of sexual reproduction. In 1924 Gimesi figured what looks like fusion of motile gametes in *Phlyctidium Eudorinae* which is followed by resting spore development from the zygote. Scherffel (1925) reported and figured the conjugation of male and female thalli in *Chytridium Characii*, *C. Spirotaeniae*, *C. Confervae*, *C. chaetophilum*, *Rhizophidium asterosporium*, *R. parasitans*, *Rhizophidium* sp., *R. goniosporium* and *R. fallax*. In the first two species long conjugation tubes are formed by the male thallus which eventually fuse with the female as in *Z. Willei*. In the other species the female thallus is figured as attached to the host cell, while the motile male gamete fuses with it. It is to be noted, however, that Scherffel does not show the actual fusion and development of the resting spores in all of these species, but merely figures such cysts with small empty adhering vesicles or companion cells which he interprets as having been the male gametes. In *Rhizophidium granulosporium*, on the other hand, the motile male gamete apparently comes to rest and germinates on the host cell, and then fuses with a motile female gamete which

becomes attached to it in this sessile condition. In 1932 Couch described "oogonia and antheridia" and their fusion to form resting spores in *Rhizophidium globosum*. In 1933 and 1935 Sparrow reported and figured an unidentified species of *Rhizophidium* in which motile isogametes germinate and infect the host cell side by side, and then develop a definite conjugation canal between them through which the contents of one moves into the other. The latter then grows in size to become the resting spore. Later in 1936 (b) he figured a somewhat similar type of sexual reproduction in *R. goniosporium* but in which the conjugation canal is not evident, confirming thus Scherffel's earlier account. In 1935 Couch described the motile male gamete in *R. ovatum* as germinating on the host cell and subsequently fusing with an active female gamete which becomes attached to it in the same fashion as Scherffel described for *R. granulosum*. The content of the minute male thallus migrates into the female gamete, nuclear fusion occurs, and a diploid resting spore is thus formed on top of the empty male gamete. In *Phlyctidium anatropum* fusion of motile isogametes or zoospores side by side is figured and described, although Couch did not observe and follow their development into zygotes or resting spores. Scherffel's, Couch's, and Sparrow's observations are confined largely to living material or thalli fixed and stained in toto and as a consequence show nothing of nuclear structure, mitosis, and very little of cell division.

In *Rhizophidium Beauchampi*, which may possibly be a species of *Phlyctochytrium* because of the presence of a sub-sporangial swelling or apophysis, Hovasse (1936) has recently shown that nuclear division in the zoosporangia is distinctly mitotic and simultaneous. While he has not described and figured the prophase stages, he nevertheless shows that the division spindle is intranuclear and oriented on two granules or centrosomes which lie inside of the nuclear membrane. He does not, however, describe cytokinesis, but merely states that it is preceded by the appearance of osmiophilic vacuoles at the poles of the nucleus.

In two recent papers (1936a and b) Sparrow has described sporangial proliferation in his new genus and species *Traustochytrium proliferum* and *Rhizophidium simplex*. In the latter species he figures a number of small incipient zoosporangia developing within the old empty ones. In view of what I have frequently observed in other rhizidiaceous chytrids I am inclined to believe that this is nothing but germination *in situ* of trapped zoospores. In *Diplophlyctis*, *Chytridium*, *Phlyctochytrium* and *Endochytrium* I have (1936b, 1937a, 1937b) often found zoospores which failed to escape and later germinated in the old sporangium. In numerous cases the germ tubes have grown down into the apophysis and rhizoidal system, and with subsequent development the thalli have attained maturity. If

only the later developmental stages of such thalli are observed one may readily get the impression that the old sporangia are proliferating. Secondary and tertiary sporangia within each other, such as I have observed in these genera, have also been described by Raitchenko (1902) in *Rhizophidium sphaerocarpum*. Previous to this time, however, Nowakowski (1877a), Rosen (1887) and de Wildeman (1895a) had reported sporangial proliferation in *Rhizidium mycophilum*, *Phlyctochytrium Zygnematis* and *Rhizidiomyces Spirogyrae*, but their accounts have not been confirmed.

True sporangial proliferation, as it has been described for *Cladochytrium*, *Nowakowskiella* and members of the Saprolegniales, is quite different from this and involves either: (1) the penetration of empty sporangia by a portion of the thallus beneath and its subsequent development into a sporangium itself; or (2) unequal and incomplete cleavage whereby a part of the nucleated protoplasm remains behind and in continuity with the remainder of the thallus and later grows and matures into an organ of reproduction. In the first category it is essential that the mycelium, rhizomycelium or rhizoids apart from the sporangia are nucleated; otherwise no proliferation could occur. As has already been noted elsewhere, however, the meager evidence in the literature on the rhizidiaceous chytrids does not support this assumption. In the few species which have been studied cytologically the nuclei are confined to the zoosporangia and resting spores and do not occur in the rhizoids.

Proliferation of the first type or category appears at present to occur only in polycentric thalli whose nuclei are variously distributed and not localized entirely in the organs of reproduction.

The second type of proliferation, on the other hand, does apparently occur in the Rhizidiaceae, and has been repeatedly described for *Harpochytrium*. While this genus does not appear to be a valid member of the chytrids it has many characteristics in common with them. The protoplasm in the lower part of the sporangium is delimited at the time of zoosporogenesis and remains undifferentiated, while that in the upper two-thirds undergoes cleavage into zoospores. The former subsequently grows and fills up the initial sporangium, and the same process is again duplicated. Gobi (1899), however, disagrees and maintains that this is nothing more than the germination and development of a zoospore which has been kept behind in the sporangium. Dangeard (1903) describes the zoosporangia as sometimes being completely emptied, and later rejuvenated to form additional secondary sporangia. This is obviously impossible when no nucleated protoplasm is left behind in the stalk or flattened holdfast and foot. Proliferation in *Traustochytrium proliferum* is apparently of the *Harpochytrium* type. According to Sparrow a large globular portion of

protoplasm in the base of the sporangium remains unchanged, while the remainder undergoes cleavage into zoospores. After these have been carried away it increases in size and becomes transformed into a secondary zoosporangium.

Extensive proliferation of the rare monocentric, rhizidiaceous thalli in *Physoderma maculare*, *P. Zeae-maydis* and *Ph. Menyanthis* has been described by Clinton, and Sparrow (1934), but neither of them indicate of what type it may be. In view of the fact that these thalli have the same organization as the true Rhizidiaceae it is improbable that the rhizoids are nucleated, and hence the proliferation may be similar to that in *Harpochytrium* and *Traustochytrium*. It is quite obvious from this discussion that the whole problem of sporangial proliferation in relation to the rhizoidal system in the Rhizidiaceae needs to be studied from fixed and stained material before any adequate conclusions can be drawn.

At this point we may perhaps best consider the two unusual saprophytic genera *Tetrachytrium* and *Zygochytrium*, described by Sorokin (1874), although there is no good evidence for including them among the Rhizidiaceae. In the latter sexual reproduction occurs by lateral conjugation between branches of the same thallus and appears to be identical in superficial appearances at least to the same process in the Zygomycetes. The thalli are thus homothallic. In *Tetrachytrium*, on the other hand, fusion between uniciliate motile isogametes occurs, forming an active biciliate zygote. The gametes come from the same sporangia and are thus not genotypically differentiated. In neither of these forms has the cytology of sexual reproduction, nuclear division, and meiosis been studied. However, in his figures of *Tetrachytrium*, Sorokin shows that cleavage in the zoosporangia is progressive from the center outwards.

It is thus obvious from this résumé that there are only three accounts of nuclear division of any significance in this large family of chytrids, while cytokinesis has scarcely been studied at all in detail. Sexual reproduction, however, has been reported in almost a score of species and exhibits a variety of differences. In the majority of species, long conjugation canals between the thalli are developed, while in others the content of the adjacent male gamete passes directly into the female through a small opening or pore. In a few species fusion between motile isogametes occurs. The cytological details of fertilization, however, such as nuclear fusion have been described in only three species, and in none of these or any member of this family has reduction division been observed. Consequently, very little conclusive evidence is known regarding the alternation of generations.

The cytology of host-parasite relationship in this family has scarcely

been studied at all in fixed and stained material, and then only indirectly. The species such as *Sporophlyctis rostrata* and *Polyphagus Euglenae* which have been investigated cytologically are completely extramatrical, except for the tips of the rhizoids, and polyphagus, and are thus less favorable for the study of this problem. The cytological data on these two forms so far relate primarily to the parasite and not the host. The majority of species in the Rhizidiaceae are quite small, evanescent, and occur chiefly on single algal cells, and it is accordingly difficult to determine the degree of pathogeneity. In many cases it is not certain from the descriptions in the literature whether the pathological conditions of the infected cells are due to the so-called parasite, or that the latter is only capable of attacking dying and dead cells.

There are, however, numerous cases on record where the effect of the parasite on the host is quite marked. In the case of parasitism on green algae the plastids are soon discolored, turn yellowish brown and red and finally degenerate as they become more or less clumped with the remainder of the protoplasm around the rhizoids or haustoria of the parasite. Sometimes marked plasmolysis may accompany such degeneration. Effects of this kind have been reported by Zopf (1887) for *Rhizophidium Sciadii* and *R. Cyclotellae* and *R. agile; Phlyctidium Bumilleriae* (Couch, 1932); *Chytridium perniciosum*, and *Podochytrium lanceolatum* (Sparrow, 1933, 1936) and numerous other workers. *Rhizophidium Sphaerotheca* and *R. pollinis*, according to Zopf (1888) cause a fatty degeneration of the protoplasm of pollen grains. Numerous other changes of a similar nature have been reported.

The reaction of the host cell wall to the presence of the parasite is often quite marked and characteristic. Braun (1855) early noted that the walls of the host cells became thickened when parasitized but he did not regard this as a protective reaction. More recently Scherffel (1925, 1926) has reviewed the literature in relation to this occurrence and shown that a great number of algae when attacked by rhizidiaceous chytrids form cellulose caps or plugs in the region of infection which prevent the entry and spread of the parasite. This is particularly evident in the case of *Chytridium Confervae* and *C. lagenula* on *Tribonema*, and *Rhizophidium fallax* on *Mougeotia*. In the former, as the rhizoids come into contact with the cross walls the host nucleus and cytoplasm become clumped together at that point and build a large conspicuous cellulose plug. Numerous other cases are cited by Scherffel where the host reaction is essentially the same. It appears nonetheless to be rather specific, since one host may react quite differently or not at all to the same or different parasites.

In contrast to the condition in the Synchytriaceae and Olipidiaceae,

however, no galls or malformations of the host tissues are produced, nor are the infected and adjacent cells stimulated to divide. *Rhizophidium fungicolum*, for instance, cause enlargement, elongation of *Gloeosporium* cells, according to Zimmermann (1902); while *Zygorhizidium* or *Ectochytridium Willei* may bring about a distinct bending of *Mougeotia* cells in addition to elongation and a slight increase in diameter (Scherffel, 1925). These changes in the host cells do not involve meresis or hyperplasia but only auxesis, cell expansion or enlargement.

CLADOCHYTRIACEAE

The meager cytological data at hand for this family are almost as conflicting as in the Synchytriaceae. The most significant contributions bearing directly on the problems of host-parasite relationship, nuclear and cell division, sexual reproduction, etc., are those of Prunet (1894), Maire and Tison (1911), Bally (1911), Tisdale (1919), Wilson (1915, 1920), Fron and Lasnier (1920), Jones and Dreschler (1920) and Bartlett (1926). Much earlier, however, Schroeter (1889, 1897) and Magnus (1897, 1901) had maintained for several species of *Urophlyctis* that the resting sporangia arose by the fusion of antheridia and oogonia. Fischer (1892) at once pointed out that what had been called the antheridia were nothing but empty vegetative "sammelzellen" such as Nowakowski (1877a) and Büsgen (1887) had shown in *Cladochytrium* and *Physoderma*, and that the so-called oogonium was only another such cell which had developed into a zoosporangium. He was confirmed by Ludi (1901) and Clinton (1902), and we now know that what the earlier students regarded as the conjugation tube in *Urophlyctis* is nothing but the isthmus connecting the resting spore with the turbinate organ cell from which it originated. Prunet, none the less, is inclined to regard the zoosporangia in *Cladochytrium viticolum* as originating from the fusion of adjacent spindle organ cells. Furthermore, in this species he describes the zoospores as being formed by the simultaneous division of the protoplasm into a number of equal masses.

Maire and Tison were the first to study cytologically the development of the thallus of *Urophlyctis Kriegeriana*, and described nuclear division in the turbinate organs and growing sporangia as strictly amitotic. In the latter structures they also observed nuclei of unusual size which degenerate and whose content aggregates in the central vacuole. No evidence of antheridia and oogonia was observed. Bally confirmed their results on this species, and in *U. Rübsaameni* also described nuclear division as amitotic. In addition he observed macro- and micronuclei, occasional fusion between the smaller ones, and nuclear multiplication by heteroschizis and

gemmation in the same manner as he, Percival, and Griggs had described in *Synchytrium*. The chromatin granules which are extruded from the nucleole during nuclear gemmation increase in size and gradually undergo hydration in the cytoplasm, and eventually the zoospores are formed around them. Thus as far as nuclear and cell division are concerned, *U. Rübsaameni* shows the same characteristics and aberrations as he had previously described for *S. Taraxaci* and *S. endobioticum*.

Tisdale showed the initial infection stages of *Physoderma Zeae-maydis*, the development of the rhizomycelium in the host cells and the germination of the resting sporangia without giving any particular cytological details of the processes. As to cell division and zoospore formation in the sporangia he merely states that "the granular content of the endosporangium begins rounding up into nuclei or oil droplets," which finally become the central bodies of the zoospores. Wilson's account of the development and cytology of *Urophlyctis Alfalfae* is comparatively unorthodox and unusual to say the least. The mature resting sporangium undergoes progressive cleavage during germination and forms both large and small zoospores which are biciliate, with one flagellum much longer than the other. These heterogamous gametes may fuse to form a motile zygote, or infect the host directly. Both the zygotes and zoospores produce amoebae, and later develop into an extended plasmodium in the host tissue, from which presumably the resting sporangia develop. No evidence of the rhizomycelium was observed. Abundant mitotic figures were seen only in the plasmodial stage. In relation to Wilson's unusual account of *U. Alfalfae* attention is also called to Scott's (1920) description of the germination of the resting spores. Each resting spore forms from one to fifteen zoosporangia which give rise to uniciliate zoospores, and is accordingly to be regarded as a prosporangium, similar in most respects to the same type of structure in *Synchytrium*. In the light of previous and subsequent descriptions of the life history of *Urophlyctis* species, it is highly probable that both Wilson and Scott were dealing with more than one organism.

Jones' and Dreschler's work on the same species appeared a little later in the same year. They showed some early infection stages, the immediate enlargement of the infected host cells, extensive replication of the rhizomycelium, and the development of the resting sporangia. Rapid amitotic division of the nuclei occurs in the turbinate organs, which is then apparently followed by cytokinesis, although nothing was observed as to the mode of this process. The organs thus become multicellular, and from these cells grow out secondary, tertiary, etc., turbinate organs, tenuous filaments and resting sporangia. No nuclei occur in the filaments except in

transit, and are confined exclusively to the turbinate organs and sporangia, in the same manner as I have described for *Cl. replicatum*. No nuclear division was observed in the latter structures, but enlarged nuclei which subsequently degenerated into the central vacuole were frequently seen.

Fron and Lasnier confirmed Jones and Dreschler as to amitosis in the turbinate organs, but in *U. Potterii* Bartlett found one typical mitotic figure in a developing turbinate organ. In the genus *Catenaria* Buckley and Clapham (1929) have shown that the resting sporangia are formed by the contraction and retraction of the contents of the zoosporangia and their investment with a thick, smooth, double-contoured wall. Occasional fusion of zoospores has been observed by the author (1934) in a saprophytic species of *Catenaria* and by Miss Ojerholm (1934) in *Physoderma Zeae-maydis*, but it is still questionable whether such fusion occurs universally and is of general significance in resting spore development.

The genera *Hypochytrium* and *Rhizidiocystis* are generally regarded as doubtful chytrids, but they have nevertheless been occasionally grouped with the Cladochytriaceae. Until more is known, however, of their structure and development they must accordingly occupy a dubious position. *Rhizidiocystis* (Sideris, 1929) is a destructive parasite in the root hairs of pineapples, being to some degree responsible for "pineapple wilt." The only distinct characteristic which it has in common with members of this family is the presence of intercalary swellings or "turbinate organs," since, according to Sideris, no zoospores are produced. *Hypochytrium* parasitizes the ascocarps of *Helotium* (Zopf, 1884) and cells of *Hydrodictyon* (Valkanov, 1929). It is likewise characterized by the presence of intercalary swellings, but possesses anteriorly ciliate zoospores. Both of these genera have only been studied in the living condition, and as a consequence nothing is known of nuclear and cell division and the cytological effects of the parasites on the hosts.

This résumé shows clearly the inadequacy and lack of cytological data for the family Cladochytriaceae. Bartlett's lone mitotic figure in a developing turbinate organ is the only one in the literature showing indirect nuclear division in this family, outside of those of Wilson in the dubious plasmodium of *Urophlyctis* and my own in *Cl. replicatum*. The presence of this figure and the abundance of regular mitosis in *Cl. replicatum* convinces me that this will undoubtedly prove to be the normal and universal mode of nuclear division in all species of the Cladochytriaceae when they have been studied intensively, although the reports of Maire and Tison, Bally, and Jones and Dreschler are to the contrary. Cell division in the zoosporangia is generally described as simultaneous when considered at all, and

as to the manner in which the turbinate and spindle organs become multicellular nothing is known, except in *Cl. replicatum*. Furthermore, there are no conclusive and indisputable accounts of sexuality.

The reaction of the host to the presence of the parasite is not particularly marked except when infected with species which are commonly assigned to the genus *Urophlyctis*. In fact, such action on the host is regarded by many mycologists as the criterion for separating this genus from *Cladochytrium* and *Physoderma*. While the author does not agree with this distinction fundamentally, it is nevertheless convenient for the purposes of this discussion. Schroeter, Fischer, Magnus, and all of the later students of *Urophlyctis* have described pronounced enlargement of the infected host cells; and when such infections occur close together and spread profusely conspicuous galls are formed. The parasite has a predilection for young meristematic cells, and infection of mature differentiated tissues occurs very seldom if at all. The infected cells do not divide, but those immediately surrounding are stimulated to rapid multiplication. This is particularly true of the vascular strand of infected growing points. As a consequence layers of thin, meristematic, and more or less isodiametric cells are formed by the host, apparently limiting the spread of the disease. These may become infected very shortly, or differentiate into vascular elements. The mature galls may thus be composed of various kinds of cells, and are the combined results of auxesis and meresis, with the latter apparently playing by far the dominant rôle.

As to the effect of the parasite on the host cell walls, nucleus and cytoplasm, there are only a few specific cytological data in the literature. Schroeter and Magnus early noted that in some species the host cell wall became greatly thickened and swollen and finally extensively perforated, somewhat similar to sieve plates, as the rhizomycelium grows through to adjacent cells. In *U. Alfalfae* particularly, Magnus described the walls as being locally absorbed in certain regions, and in others forming conical and coralloid cellulose plugs which extend out into the lumen of the cell. Bally also noted a thickening of the cell walls of *Rumex scutatus* when parasitized by *U. Rübsaameni* but no cellulose plugs. On the other hand, such thickened and swollen walls showed no cellulose but rather a marked pectic reaction with various microchemical tests. Bally is thus of the opinion that the first step in the breakdown of the wall is the secretion of an enzyme by the fungus which dissolves the cellulose and leaves only a pectic residue. However, many years previously Saccardo and Mattirolo (1895) had claimed that the walls of infected cells of alfalfa were typically cellulose in composition, while Lagerheim (1898) maintained that they became lignified. Bartlett supports Lagerheim's contention for *Lotus corniculatus*

when parasitized by *U. Potteri*. Jones and Dreschler, on the other hand, noted only a thickening of the wall, but made no tests as to its fundamental composition. Walls of adjacent enlarged cells may be dissolved or absorbed, so that the replicated rhizomycelium may often lie in a multinucleate symplast, according to Bally. The nucleus of the host cell usually enlarges considerably and may become quite irregular in outline. The latter change, however, is not as marked as in the case of cells infected with *Synchytrium*. The increase in size, on the other hand, may not be entirely due to the presence of the parasite, but primarily to an effort to maintain the nucleo-cytoplasmic ratio in the enlarging cell. Magnus, Bally, and Jones and Dreschler, however, believe it is chiefly due to the former. The nucleoli enlarge and multiply, become intensely basophilic, and are the last of the nuclear elements to disintegrate, while the chromatin and linin become more sparse and gradually disappear. Very little definite is known about the effect on the host cytoplasm. None of the students of *Urophlyctis* discuss this problem, but Jones' and Dreschler's figures indicate that it becomes highly vacuolated as the infected cells enlarge.

The majority of species of *Cladochytrium*, *Amoebochytrium*, and *Catenaria* are saprophytic, and for those which are weakly parasitic little or nothing is known about their effect on the host. On the other hand, *Cl. viticolum*, according to Prunet (1894), is a destructive parasite of grape vines and causes definite swellings and spots in the infected regions. It is not certain, however, from his descriptions whether these galls are the result of auxesis or meresis or a combination of both. On the basis of its effect on the host this species will perhaps be transferred to *Physoderma* if Minden's (1911) classification is widely accepted.

The species of *Physoderma* are parasitic and cause only slight enlargement and discoloration of the host tissues, according to Minden's (1911) limitations of the genus. As noted above, they have been studied somewhat extensively from the structural standpoint, but none of these studies have contributed much to our knowledge of the cytology of host-parasite relationship. The contributions of Schroeter, De Bary (1874, 1884), Büsgen, Clinton and Tisdale on various species of this genus are notably lacking in this respect.

SUMMARY

1. The zoospores of *Cladochytrichum replicatum* penetrate the host cell by a small germ tube into which the nucleus migrates. It usually comes to rest near the tip of the tube, and the latter then enlarges in that region and becomes the initial uninucleate spindle organ. One or more tenuous

filaments and rhizoids develop from this organ and pass into adjacent cells. The nucleus divides very shortly, and one and occasionally both daughter nuclei migrate out into these filaments and thus secondary and tertiary spindle organs or "sammelzellen," etc., are established. Accordingly, as this continues the thallus is continually replicated as it spreads in the host tissue and soon becomes polycentric in structure and organization. Its development and replication is thus distinctly homoeotic.

2. Rhizoids are abundantly developed on the tenuous filaments and often from the surface of the spindle organs and zoosporangia and soon run out to fine points and end shortly. They appear to be the primary organs of absorption.

3. The nuclei in mature thalli are localized in distribution and confined to the spindle organs, zoosporangia and resting spores. They may be found in the tenuous filaments only in transit or during migration to establish successively new centers. They are thus not more or less evenly distributed along the entire length of the thallus as appears to be the condition in the mycelium of the higher fungi. Doubtless as a result of such localization of the nuclei in the spindle organs during the growth period, these enlargements become the vegetative centers of thallus replication.

4. The thallus or rhizomycelium of *C. replicatum* at maturity thus consists of rhizoids, tenuous filamentous portions, spindle organs, zoosporangia and resting spores. The so-called tenuous filaments connecting the more or less globular structures vary considerably in diameter and may become quite large under certain conditions. The rhizomycelium possesses many characteristics in common with rhizidiaceous thalli of the family Rhizidiaceae and the mycelium of the higher fungi, and as far as our knowledge of the chytrids goes at the present time appears to stand more or less midway between the two. On one hand it develops numerous enucleate rhizoids, and on the other hand it is polycentric in structure and organization. There is, however, no good evidence at hand to show that it is a transitional evolutionary type of thallus which has evolved from the rhizidiaceous forms and then in turn given rise to a true mycelium.

5. Nuclear division in the spindle organs and zoosporangia is mitotic with well-defined achromatic spindle and chromosomes. The resting nucleus possesses a large nucleole and a faint chromatin reticulum oriented to some degree on the former. During the prophase changes the reticulum becomes denser and more basophilic, but the nucleole does not undergo much change in size, shape, and staining capacity. No conspicuous nucleolar discharges of chromatin have been observed, and at present it is not certain that the large nucleole is to be regarded as karyosome. The chromosomes are quite small, and in cases of faulty fixation and staining may

become indistinct as individual bodies. The number falls between six and nine. The division spindles are intranuclear but their origin is obscure. Some evidence of centrosomes and astral rays has been found, but the data at hand are not sufficient to warrant the general conclusion that they are universally present. So far they have not been found to play any part in the origin of the spindle and the division of the cell. In the late anaphases and telophases the daughter chromosomes usually fuse into two compact, optically homogeneous oval masses which have an appearance strikingly similar to the nucleoli of the resting nucleus and eventually become surrounded in some unknown manner by a membrane. The nucleole of the mother nucleus generally retains its size and staining reaction during division and finally with the disappearance of the nuclear membrane is liberated into the cytoplasm where it gradually diminishes in size and disintegrates.

6. Cytokinesis occurs after nuclear division has been completed and is independent of the activity of the spindle fibers or other similar kinoplasmic material. In the spindle organs circular clefts or furrows develop centripetally and within them are formed delicate plates or membranes which gradually progress with the clefts toward and meet in the center of the cell. In the zoosporangia cell division is accomplished by progressive cleavage. In non-vacuolated sporangia the cleavage furrows begin at the periphery and progress more or less radially between the nuclei until the protoplasmic mass is cut up into polyhedral segments. Cleavage may be accompanied by shrinkage, but after the process is completed the segments may rehydrate and fill up the zoosporangium again. The lines of demarcation thus become finer and less distinct, and the sporangium frequently has the appearance of having undergone simultaneous fragmentation. In sporangia with large central vacuoles the cleavage furrows usually start at the boundary of the vacuole and progress centrifugally. In a few instances both centripetal and centrifugal cleavage have been observed within the same sporangium.

7. During the process of cleavage and the maturation of the segments the nucleole usually diminishes in size, and numerous basophilic granules or bodies appear in the cytoplasm near the nuclear membrane at the pole opposite the nucleole. Whether these are chromidia extruded from the nucleole during its diminution in size or chondriosomes has not been definitely determined, but they nevertheless appear to increase in size and volume and eventually fuse to form a dense staining extra-nuclear cap. This varies considerably in size but usually sits as a more or less hemispherical hood over the apex of the nucleus. The mature zoospores possess thus a well-defined nucleus with a small nucleole, a conspicuous nuclear cap

and a single posteriorly attached cilium. The point of attachment of the cilium and the nuclear membrane are frequently connected by delicate cytoplasmic strands.

8. No fusion of motile or quiescent gametes has so far been observed. From the data at hand the more or less spherical, hyaline resting spores appear to be formed asexually. They are predominantly uninucleate, although occasional bi- and tetranucleate spores may be formed. Their method of development seems to be similar to that of the evanescent zoosporangia, with the exception that the golden-red globules are not conspicuously developed and nuclear division is ordinarily suppressed. As the spore grows in size the more or less hyaline globules fuse to form a large central one, and the wall begins to thicken and soon attains mature proportions.

9. The Chytridiales in general have been but little studied cytologically. Less than two dozen species have been investigated, and very little is known of the processes of sexual reproduction, nuclear division, cytokinesis, etc., in the group as a whole. Amitosis, nuclear budding and fragmentation, and the origin of nuclei from granules in the cytoplasm has been described in a large number of species, while cell division is reported to occur by cell plate formation, simultaneous fragmentation of the protoplasm, and progressive cleavage. Chromosome counts have been made in only seven species, and there are scarcely half a dozen detailed accounts of indirect nuclear division for the whole group. While sexual reproduction has been observed in a large number of species, meiosis has been found and described in only one species.

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Explanation of Plates

All figures refer to *Cladochytrium replicatum* and were drawn from fixed and stained preparations with the aid of a Spencer camera lucida, a Zeiss 2 mm. N. A. 1.30 apochromatic objective, and compensating oculars nos. 6 and 8.

Plate 1

Fig. 1. A zoospore with a large nuclear cap lying on the surface of an epidermal cell of *Panicum variegatum*.

Fig. 2. An early germination stage.

Fig. 3. A later stage showing the migration of the nucleus into the elongated germ tube.

Fig. 4. A very young thallus showing the primary pear-shaped nucleus in the initial spindle organ.

Fig. 5. Equatorial plate stage of division of the primary nucleus in the initial spindle organ.

Fig. 6. A primary two-celled spindle organ showing an early stage of the migration of a daughter nucleus into the tenuous portion of the rhizomycelium.

Fig. 7. A slightly later stage in which the apex of the nucleus is drawn out into a densely stained rod.

Fig. 8–10. Nuclei in transit to secondary spindle organs.

Fig. 11. A secondary spindle organ with a pear-shaped nucleus. The nucleole is in the form of a broad circular band, and from the pointed end of the nucleus runs a conspicuous cytoplasmic strand which may possibly indicate the path taken by the nucleus in entering.

Fig. 12. A portion of a rhizomycelium showing the primary and secondary spindle organs, rhizoids, and habit of branching and variations in diameter of the filamentous portions.

Plate 2

Fig. 13. A secondary spindle organ showing the structure of the resting nucleus.

Fig. 14. A median section of an enlarged resting nucleus showing the structure of the chromatin reticulum. The nucleole was not visible in this section.

Fig. 15, 16. Surface and side views of resting nuclei with circular band-shaped nucleoli.

Fig. 17. An early prophase nucleus from poorly fixed and overstained material. The chromatin reticulum is highly shrunken and densely stained.

Fig. 18. A similar stage from the same type of material, but less densely stained. The reticulum is slightly oriented on the nucleole.

Fig. 20. A very early prophase stage in well-fixed and stained material. The reticulum is fairly dense, while the nucleole has an uneven contour and appears to be composite.

Fig. 21. A binucleate zoosporangium with both nuclei in the spireme stage of division. In the right-hand nucleus the spireme bands appear coiled or zigzag in appearance and are definitely oriented on the nucleole. On the membrane of each nucleus lies a densely stained body with cytoplasmic strands radiating from it, which suggests the presence of centrosomes and astral rays.

Fig. 22. A later prophase nucleus showing the shortening and thickening of the spireme strands.

Fig. 23. A slightly later stage showing three small and three larger discrete bodies. The nucleole lies at the left side in the shape of a ring.

Fig. 24. A late prophase nucleus with seven discrete bodies or chromosomes. The achromatic spindle appears to be forming.

Fig. 25. A very late prophase stage in the primary nucleus of a zoosporangium. The intra-nuclear spindle has been fully formed, and the chromosomes are being drawn into the equator of the nucleus. The poles of the spindle converge on two densely stained bodies lying on the nuclear membrane, and from the latter radiate faint irregular cytoplasmic lines.

Fig. 26. An equatorial plate stage in which the chromosomes appear more or less merged into an irregular band across the equator of the spindle.

Fig. 27. A similar stage showing a broad spindle with the chromosomes arranged in a ring around its periphery.

Fig. 28. Polar view of an equatorial plate stage showing six chromosomes arranged in a flat loop. The nucleole is irregular in contour and somewhat cup-shaped.

Fig. 29. A two-celled spindle organ showing the division of the remaining nucleus. One pole of the distorted spindle is centered on the nucleole.

Fig. 30. An early anaphase stage. The triangular-shaped nucleole lies at one side and has apparently distended the nuclear membrane in that region.

Fig. 31. A slightly later stage in which, due to overstaining, the two chromosome groups appear as homogeneous, irregular curved bands.

Fig. 32. An anaphase stage with a narrow intra-nuclear spindle.

Fig. 33. A later stage in which the individual chromosomes are fairly distinct. The nucleus is surrounded by a dense mass of granules which tend to obscure the nuclear membrane.

Fig. 34. A late anaphase stage in which the chromosomes have arrived at the poles of the spindle.

Fig. 35. A later stage in which the nuclear membrane has almost completely disappeared. The spindle has elongated considerably; the nucleole lies free, and the chromosomes have united into two compact groups.

Fig. 36. A further anaphase stage. The elongated spindle is represented by a few strands and is slightly bent, while around the chromosome groups are distinct polar radiations. The nucleole is still present in the cytoplasm as a densely basophilic globule.

Plate 3

Fig. 37. An early telophase stage, perhaps, in which the spindle is still faintly evident. The chromosomes have united into two compact, optically homogeneous groups, and above them appear clear spaces partly surrounded by a faint boundary which suggests the origin of the nuclear membrane. The edges of the nucleole are drawn out along the spindle.

Fig. 38. A spindle organ showing the completion of nuclear division. The daughter chromosome groups look like two large nucleoli, while the old nucleolus lies in the cytoplasm. No well-defined nuclear reticulum is visible.

Fig. 39. Doubtless a later stage showing the diminution in the size of the old nucleole and the development of a reticulum in the daughter nuclei.

Fig. 40. A spindle organ showing the two daughter nuclei lying close together in the region formerly occupied by the mother nucleus. The old nucleole lies as a disintegrating mass at the right side.

Fig. 41. An early stage of cell division in which two faint furrows or clefts are visible.

Fig. 42. A further advanced stage showing the faint membranes which have been laid down in the advancing grooves.

Fig. 43. A later stage of cytokinesis.

Fig. 44. Cytokinesis has been completed.

Fig. 45. An elongated unicellular spindle organ with four nuclei in which karyokinesis has apparently not been followed immediately by cell division.

Fig. 46. An odd-shaped spindle organ with one of the nuclei dividing at right angles to the long axis.

Fig. 47. A five-celled spindle organ, two of whose cells have developed into zoosporangia.

Fig. 48. A two-celled spindle organ with a zoosporangium developing directly from the surface of one of its cells.

Plate 4

Fig. 49. A bi-nucleate zoosporangium showing proliferation.

Fig. 50. A cross section of a four-nucleate zoosporangium, all nuclei of which are in the prophases of division.

Fig. 51. A longitudinal section of a similar structure with the three visible nuclei in the equatorial plate stage of division.

Fig. 52. An eight-nucleate zoosporangium in longitudinal section with six nuclei in division.

Fig. 53. An oval zoosporangium with a large central vacuole.

Fig. 54. An early stage of progressive cleavage in a medium-sized sporangium.

Fig. 55, 56. More advanced stages of cleavage in small zoosporangia.

Fig. 57, 58. Small and large sporangia showing the completion of progressive cleavage and the presence of large masses of densely basophilic granules around the nuclei.

Fig. 59. A peripheral cleavage segment showing the position of the nucleus and the small nucleole.

Fig. 60. A cleavage segment showing five densely basophilic bodies in the cytoplasm.

Fig. 61. A cleavage segment showing an increase in the number of such bodies and their aggregation at the pole of the nucleus opposite the nucleole.

Fig. 62. A later stage of aggregation and fusion of bodies to form the extra-nuclear cap.

Plate 5

Fig. 63. A mature sporangium with zoospore initials whose nuclei appear as clear spaces surmounted by densely stainable nuclear caps.

Fig. 64A, 64B, and 65. Zoospores showing the variations in size and extent of the nuclear cap.

Fig. 66. An early stage in resting spore formation.

Fig. 67. A later stage showing the increased vacuolation of the cytoplasm and thickening of the cell wall.

Fig. 68. A bi-nucleate incipient resting spore.

Fig. 69. A similar spore which has apparently been formed from a young zoosporangium, whose exit tube is still present.

Fig. 70. A four-nucleate resting spore.

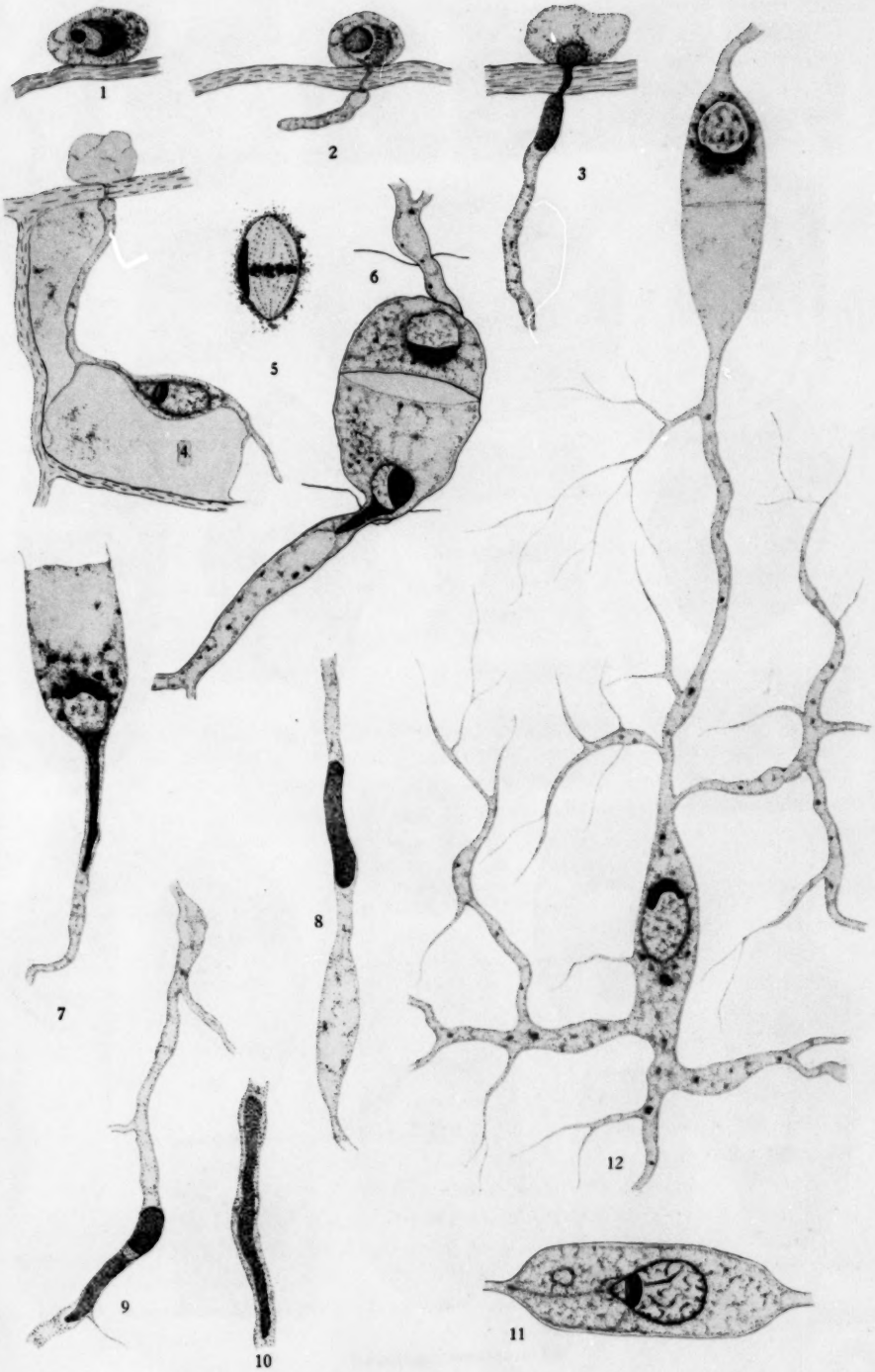
Fig. 71. A two-celled resting spore formed from an encysted spindle organ.

Fig. 72. An almost mature resting spore which has apparently developed from a young uninucleate zoosporangium. The exit tube of the latter is still intact.

Fig. 73, 74. Mature resting spores showing the flattened nucleus from the side and above.

Plate 6

Showing the structure, cytology, distribution and organization of the rhizomycelium of *C. replicatum* in a portion of an internode of *Nitella flexilis*.



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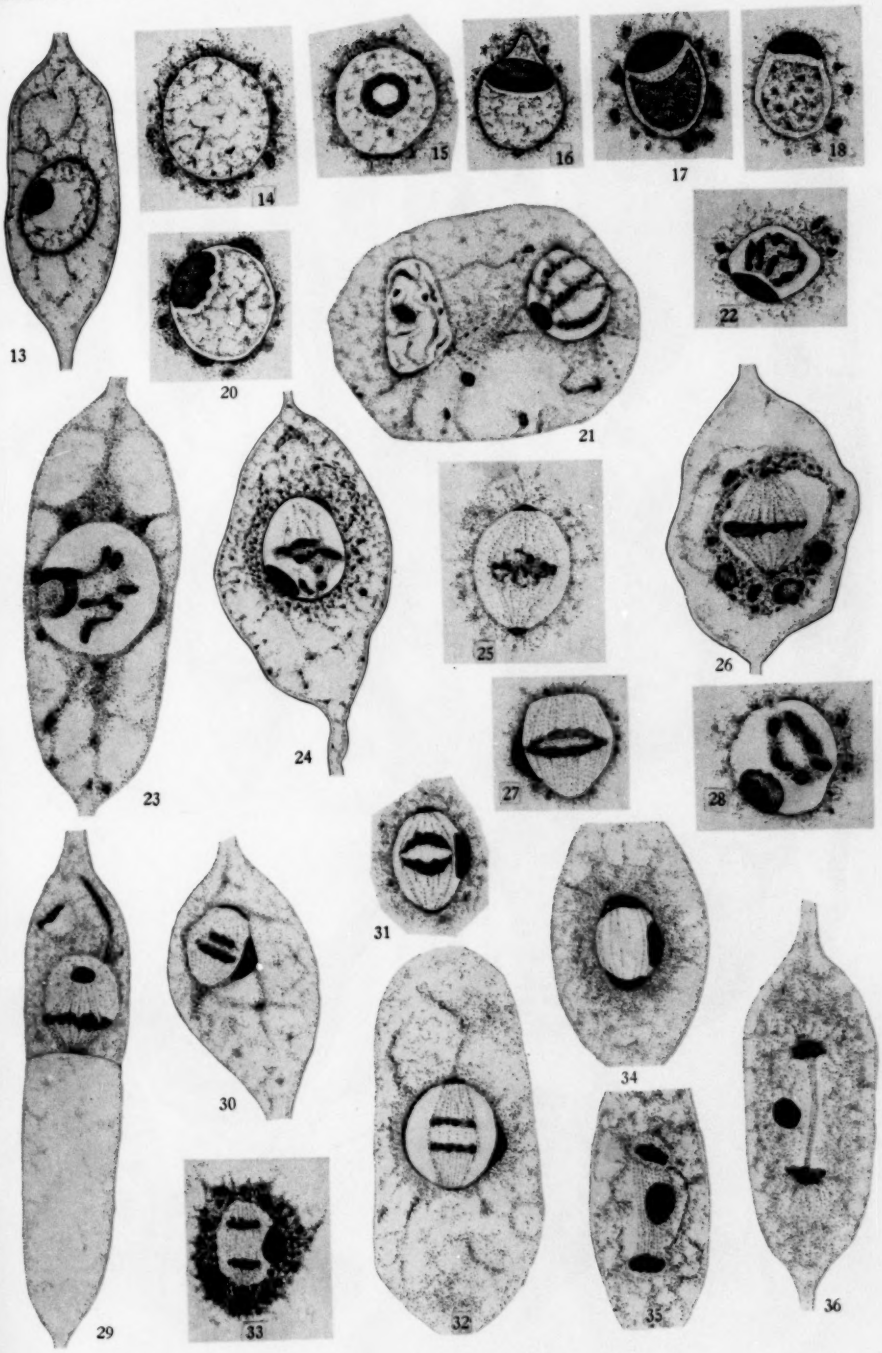

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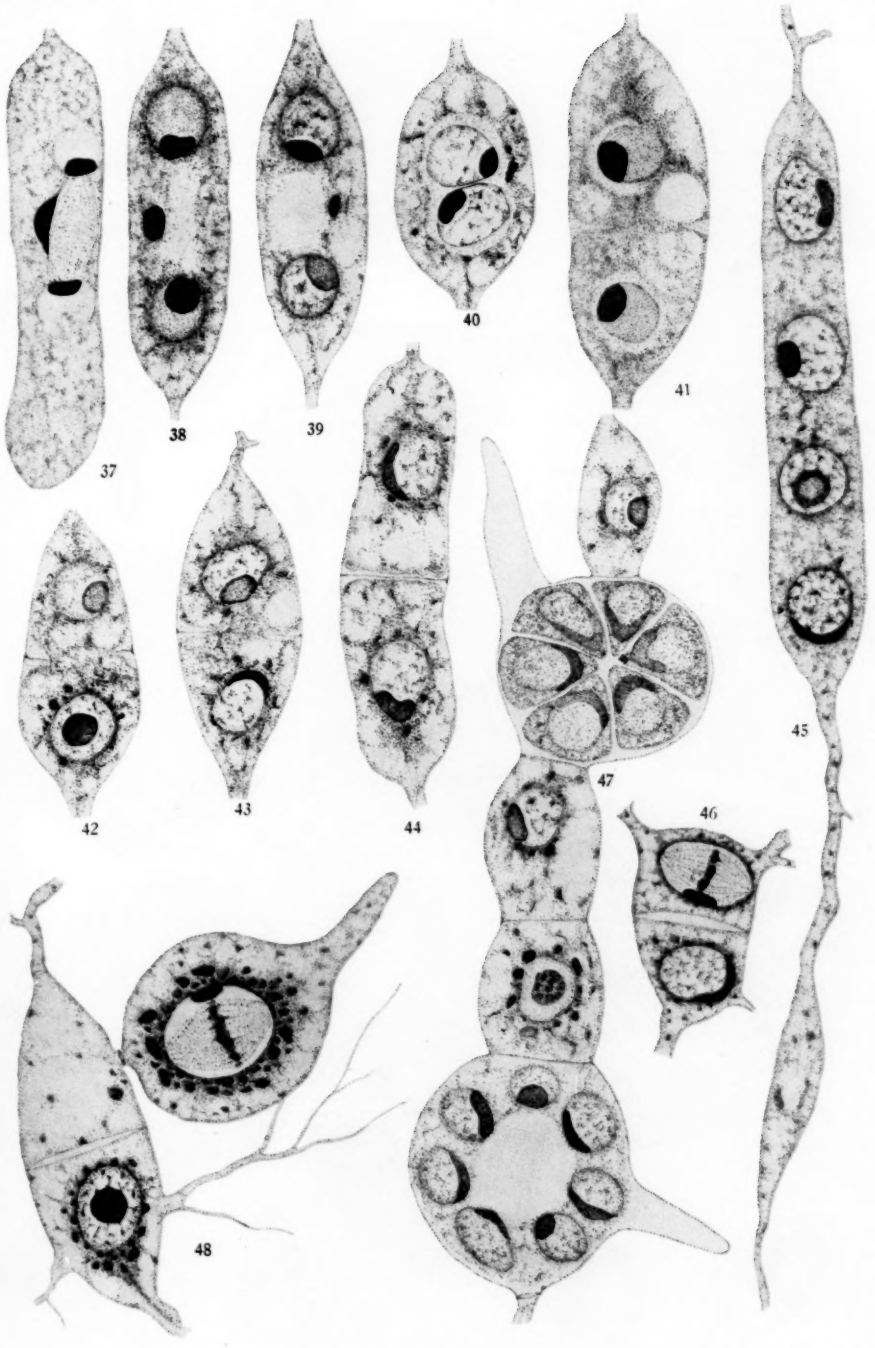

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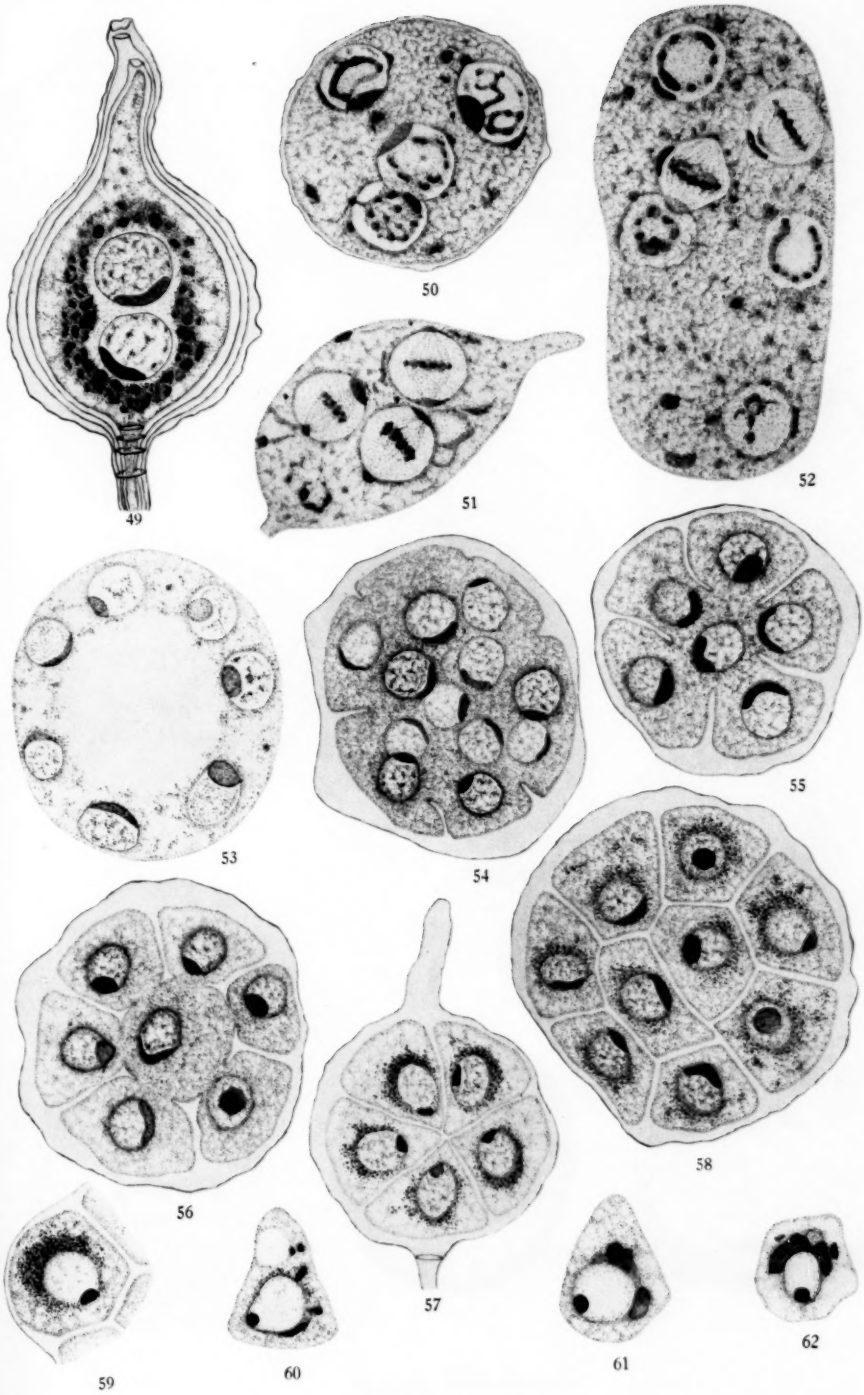

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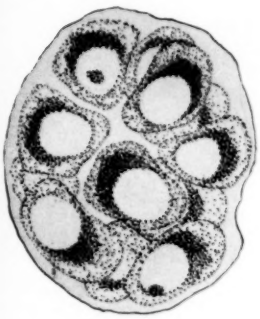

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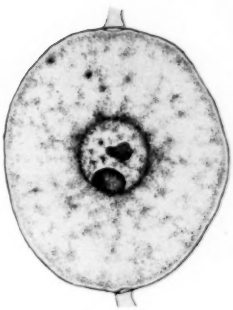

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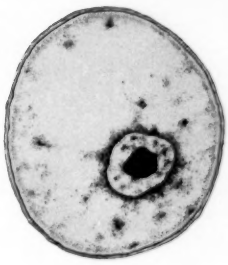

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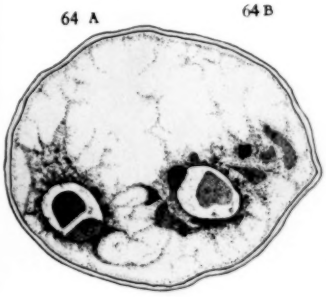
64 A

64 B



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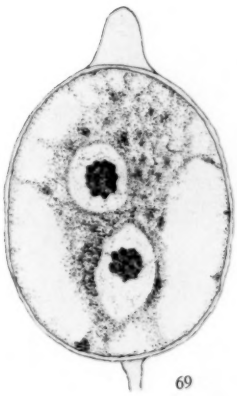

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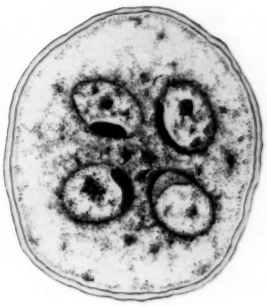

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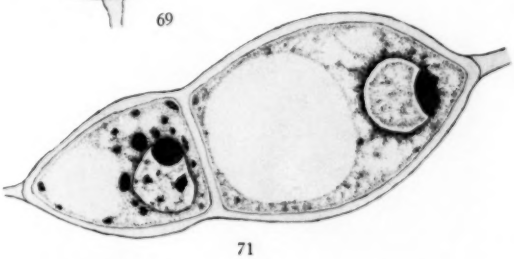

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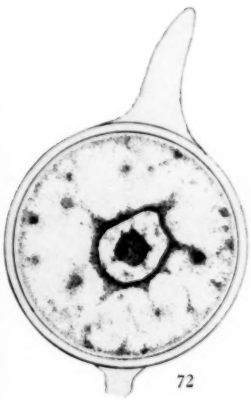

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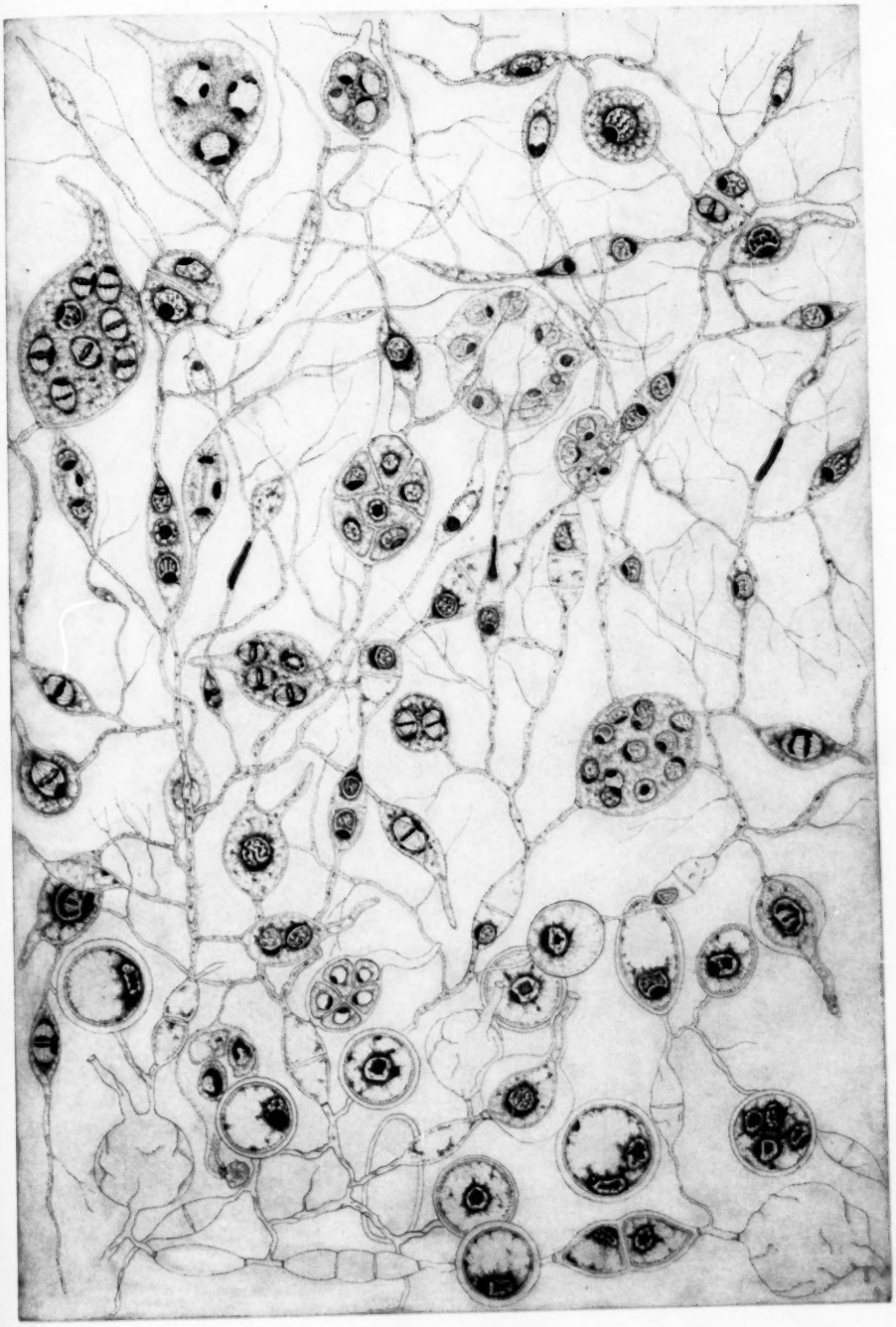

KARLING: CHYTRIDIALES